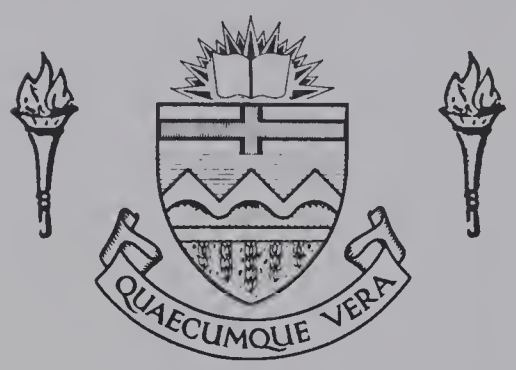


The University of Alberta
Printing Department
Edmonton, Alberta

THE UNIVERSITY OF ALBERTA

DEVELOPMENT OF ANTIMACROPHAGE SERA: STUDIES ON PROLONGATION OF ALLOGRAFT SURVIVAL

by

© REX WENDELL JORDAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

SPRING, 1971

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Development of Antimacrophage Sera: Studies on Prolongation of Allograft Survival" submitted by Rex Wendell Jordan, in partial fulfilment of the requirements for the degree of Master of Science (Surgery).

ABSTRACT

In recent years, the experimental transplantation of segments of small intestine has been gaining increasing popularity. Up to the present time, however, there have been relatively few experiments performed on the use of immunosuppressive agents to prolong the survival of such allografts. It has recently been reported that antimacrophage serum possesses an immunosuppressive potency comparable to that of the better characterised antilymphocyte serum.

We have attempted to compare the effects of antimacrophage serum on the prolongation of survival of canine intestinal allografts with the results obtained employing three other immunosuppressive regimens: antiperitoneal cell serum ('impure' antimacrophage serum), antilymphocyte serum and a combination of azathioprine, prednisone and antilymphocyte serum.

Our results have demonstrated a very significant prolongation of survival in dogs treated with antilymphocyte serum and those treated with a combination of antilymphocyte serum, azathioprine and prednisone. There was no prolongation of survival in dogs treated with antimacrophage serum and the results obtained with antiperitoneal cell serum are of doubtful significance and will require further investigation to ascertain their significance.

ACKNOWLEDGEMENTS

The completion of this work has depended upon the expert help of people of various disciplines. It is impossible to mention every one, but the author has been particularly fortunate in receiving the skilled assistance of the following:

Doctor P.A. Salmon: whose continued guidance as a supervisor was always a source of inspiration.

Doctor J.C. Russell: who performed the statistical analysis of results.

Mr. Clarence Moisey: whose assistance made the day to day chores simple.

Mr. Alvin Todd: for technical assistance far beyond the call of duty.

Miss Barbara Britton and Mrs. Janet Larsen: who painstakingly typed the manuscript.

Mrs. M. McCubbin: for her help in the preparation of antisera.

The Staff of the University of Alberta Photo Services: whose cordiality and speed in making numerous prints were exemplary.

The Edmonton Civic Employees Welfare Chest Fund: whose generosity made this research financially possible.

TABLE OF CONTENTS

DEVELOPMENT OF ANTIMACROPHAGE SERA: STUDIES ON PROLONGATION OF ALLOGRAFT SURVIVAL

LIST OF TABLES[1]

DEVELOPMENT OF ANTIMACROPHAGE SERA: STUDIES ON PROLONGATION OF ALLOGRAFT SURVIVAL

[1]Tables 1-5 survival of intestinal allografts (days) in dogs on immunosuppressive regimens.

LIST OF ILLUSTRATIONS

DEVELOPMENT OF ANTIMACROPHAGE SERA: STUDIES ON PROLONGATION OF ALLOGRAFT SURVIVAL

FIGURE PAGE

FIGURE PAGE

CHAPTER I

LITERATURE REVIEW

I. Statement of the Problem

The work of Medawar[1] has established the fact that the rejection of tissue and organ allografts is an immunological response mediated by cellular elements. At this time it appears that the most important cell type is the lymphocyte. The search for immunosuppressive agents, capable of producing long-term survival of grafted organs with minimal toxic side effects, has caused investigators to use sera developed specifically against the lymphocyte. The results obtained through the use of anti-lymphocyte serum alone in suppressing the rejection of whole organ grafts have been encouraging. Graft survival has been prolonged, although not for indefinite periods, and toxicity appears to be less than in the case of some of the chemotherapeutic agents. However, it is now apparent that antilymphocyte serum is not the complete answer to cellular rejection, and that other solutions must be sought.

Recent experimental work suggests that a second cellular element, the macrophage, may have an important rôle in mediating the rejection response. The macrophage, in the afferent phase of the immunologic "reflex", is thought to act by processing foreign antigen and transferring immunogenetic information to the lymphocyte. Furthermore, its

presence is required for lymphocytic cellular transformation which occurs in the presence of foreign antigen. In the efferent phase, the macrophage may effect target cell death. The apparent importance of the macrophage in the rejection phenomenon has resulted in a search for methods of selectively modifying the rôle of this cell.

These methods have included the use of specific macrophage toxins such as silica,[2] and the development of antisera specifically directed against the macrophage. In most instances, peritoneal exudates have been the source of antigen used in the preparation of antimacrophage sera. One group of investigators has reported the development of an effective antimacrophage serum, the use of which has resulted in a significant prolongation of allograft survival. However, the method used by this group for harvesting macrophages is probably not sufficiently selective, for it is known that in addition to macrophages, the cellular fraction of peritoneal exudates contains a considerable number of lymphocytes. Therefore, the serum produced following injection of the mixed peritoneal cells would probably have both antimacrophage and antilymphocyte properties.

We have thus attempted to:

1) obtain a purified suspension of macrophages,

2) prepare a potent antiserum, to this suspension (henceforth referred to as 'pure' antimacrophage serum),

3) investigate the in vitro activity of this antiserum, and

4) compare its effects on the prolongation of survival of intestinal allografts with those obtained, using other immunosuppressive

sera and agents:

a) antilymphocyte serum developed in goats against dog lymph node lymphocytes,

b) a combination of antilymphocyte sera and the standard immunosuppressives, azathioprine and prednisone,

5) to prepare an antiserum to the unmodified cellular fraction of peritoneal exudates, resulting from introduction of sterilised olive oil into the peritoneal cavity of dogs and to determine the effects of this antiserum (henceforth referred to as 'impure' antimacrophage serum) on the survival of intestinal allografts.

II. Introduction

In recent years, transplantation of tissues and organs has gained increasing popularity as a treatment of certain diseases which are both incurable and refractory to more conventional methods of medical therapy. Though surgical techniques have developed to a point where organ transplantation, rather than remaining a dream of the future, is now technically feasible, allograft rejection is still the major unsolved problem of transplantation surgery. During the past decade, the application of available surgical and immunosuppression techniques has met with surprising success. However, it seems evident that if both allo- and heterografting are to be applied with uniform success on the widest possible scale, other techniques of suppressing rejection or altering host response to grafts must be found. The solution to the problem must lie in greater understanding of the basic cellular and immunologic processes involved. These problems have been under scrutiny for many years, and a considerable volume of data is already at hand.

Loeb (1902)[3] compared the survival of similar tissues after auto- and allotransplantation. He noted that the former survived indefinitely, and the latter were invariably destroyed. The destruction of the allograft was accompanied by a marked inflammatory reaction.

The classic studies of Medawar[1] first established that the lymphocyte had a specific rôle in rejection, and resulted in the concept of cell mediated immunity. In rabbits, he compared the cellular response to skin allografts with that resulting from the Arthus reaction. The main difference was in the nature of the accompanying cellular infiltrate, predominantly mononuclear in the former, mainly granulocytic in the latter. However, he failed in an attempt to transfer transplantation immunity with serum, confirming that a cell mediated process was involved rather than one involving humoral antibody production.

Later Mitchison[4] was successful in transferring immunity against an allogenic mouse lymphosarcoma with isogenic lymph node cells from sensitised donors. Billingham et al.[5] were similarly successful in transferring skin allograft immunity in mice with lymph node cells.

Later investigations by Waksman[6] and Weiner, Spiro and Russell[7] confirmed that a mononuclear cell infiltration occurs prior to the appearance of degenerative changes in the epithelial and other cellular elements of the graft. Waksman concluded that invading mononuclear cells had a direct cytopathogenic action on antigen containing cells in vessel walls and throughout the graft.

There is now much evidence confirming the thesis that the infiltrating host mononuclear cells do, in fact, represent a specific immunologic effector mechanisms in operation. This evidence has been elegantly summarised by Billingham,[8] who divided his summary into five categories:

1) evidence from histological studies on allografts,

2) evidence from in vivo experiments,

3) evidence from diffusion chamber experiments,

4) evidence from in vitro experiments with sensitised lymphocytes, and

5) evidence from in vitro experiments with unsensitised lymphocytes.

He gave four postulates as to the manner in which the cyto-pathogenic effect of lymphocytes is mediated:

1) the cells synthesize an antibody that is normally released only when they are in intimate contact with homologous target cells,

2) the cells do not synthesize antibody but carry on their surface an effective, cell bound antibody having a specific affinity for cells (cytophilic antibody) and which is synthesised elsewhere in the body,

3) the cells synthesize a special kind of antibody which is permanently incorporated on their surface and is only effective when an intimate contact with a target cell is established, and

4) antibody is not the ultimate effector or mediator of graft destruction. Following some kind of 'recognition' event which has an immunologic basis and requires an intimate relationship between sensitised cell and graft antigen (cellular or sub-cellular), the sensitised lymphocytes release or secrete and/or cause other host cells to release some nonspecific pharmacologically active agent, analogous to histamine, which is the ultimate effector.

Immunological responses are presently divided into those medi-

ated by cells and those mediated by humoral antibody. Both types of response are mediated by the small lymphocyte,[9,10] a cell whose precursors are from bone marrow.[11,12] The development of cell mediated immune responses as well as the humoral antibody response to certain antigens, is abolished by neonatal thymectomy in mice and rats.[13-18] In birds, humoral antibody production, but not cell mediated reactions, is dependent upon the bursa of Fabricius, and it has been suggested that gut associated lymphoid tissue provides the mammalian analogue.[19] This suggests the presence of two distinct lymphocyte populations, one dependent on the presence of the thymus and the other independent of the thymus. They differ in their distribution and life span; the thymus dependent lymphocytes constitute the greater part of the recirculating pool of small lymphocytes[20] and have a relatively long half-life, whereas the population of lymphocytes, independent of thymic sponsorship, is more restricted to lymphoid tissue and appears to be short-lived.[21]

The structural changes in lymphoid tissues occurring in response to antigenic stimulation have been studied in great detail.[22-26] In essence these consist of transformation of lymphocytes into large rounded cells having large vesiculated nuclei and prominent nucleoli and pyroninophilic cytoplasm. These are the so called 'blast' cells, and increasing development of the endoplasmic reticulum, reduction in nuclear volume, reduction in mitochondrial size, and extensive development of the Golgi complex, leads to the development of a cell eminently suited for antibody production. This is the plasma cell. However,

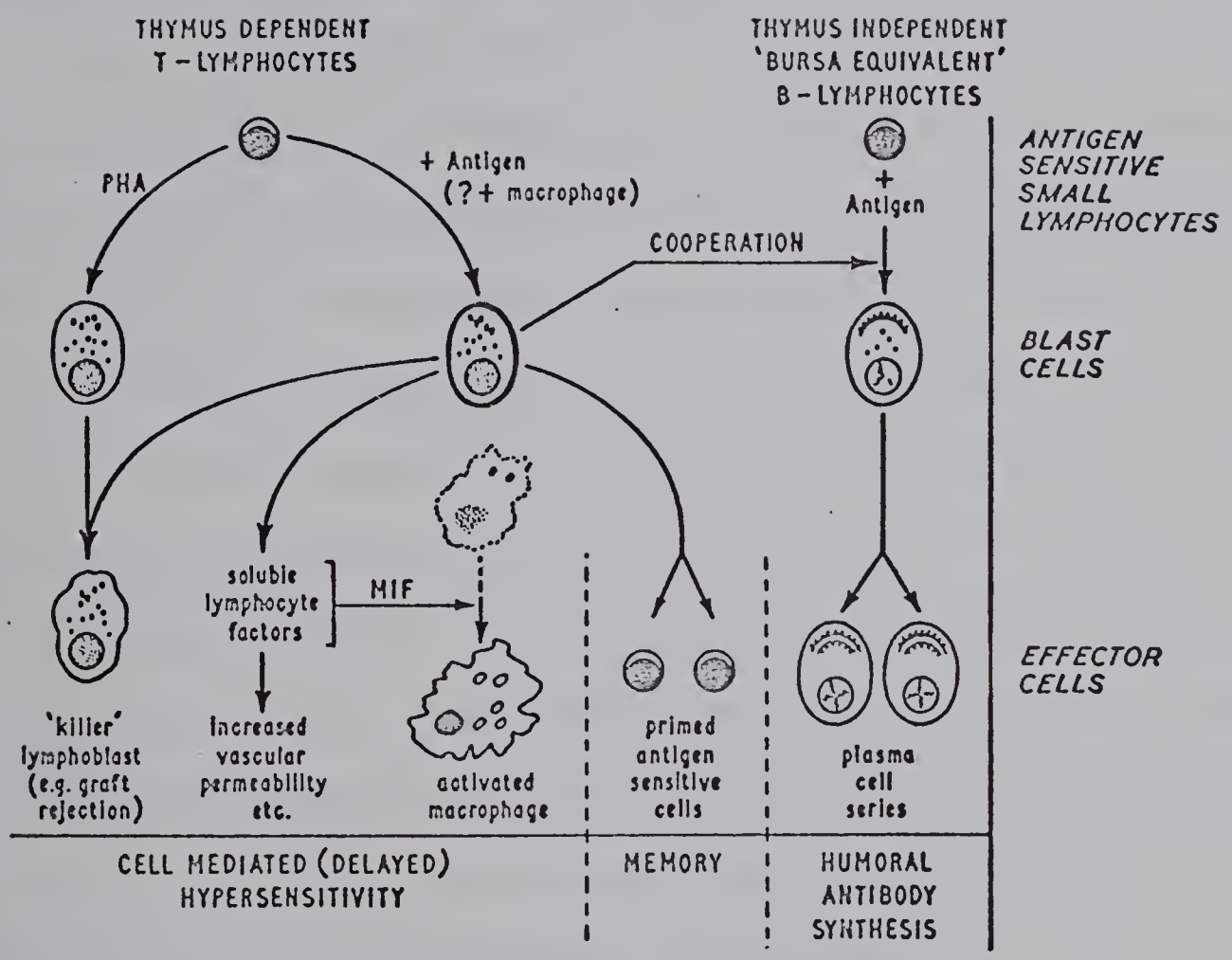


Fig. 1.-- Diagram of the role of thymus dependent (T) and bursa equivalent (B) lymphocytes in immunologic responses. (ROITT, I.M., GREAVES, M.F., TORRIGIANI, G., BROSTOFF, J., and PLAYFAIR, J.H.L. Lancet, 2:367, 1969.)

other cells may produce antibody.[27]

Lymphocytes from individuals sensitised to a given antigen are transformed in culture on contact with the antigen, probably through combination involving a specific antibody on the surface.[28] The presence of surface immunoglobulins on lymphocytes has been indicated by the experiments of Sell[29] and Sell and Gell.[30] Intimate contact between lymphocytes and macrophages involving cytoplasmic interaction is required for lymphocyte transformation in the presence of a foreign antigen.[31-41] Thus, lymphocyte preparations which have been largely freed of phagocytic cells are unresponsive to antigen. However, activity can be restored by the re-introduction of macrophages.[42,43] The rôle of the macrophage is probably one of 'processing' of antigen, a step that may be necessary before transformation can occur. In certain instances antigenicity may even be increased as when bovine serum albumin is the antigen.[44] The association between these two cell types is very intimate and involves cytoplasmic interaction.[38]

The requirement that antigen be processed by macrophages is not, however, universal and in the case of certain antigens, (e.g. keyhole limpet hemocyanin), antigenicity is greatly reduced after uptake into peritoneal macrophages.[45]

The transformed lymphocyte has cytotoxic properties for cells to which it is sensitised.[46,47,48] The exact nature of its destructive action is not known.

The interaction of antigen with sensitised lymphocytes leads

to the release of soluble factors. One of these factors inhibits the migration of macrophages in tissue cultures and has been called the "migration inhibitory factor".[49,50] The in vivo effect of this latter factor would be to immobilise wandering macrophages at the site of reaction with antigen where they might exert a cytotoxic action or scavenge cells already damaged by lymphocytes.

Of the heterologous antisera presently under investigation, antilymphocyte serum is by far the best characterised and comparison of its properties with those of antimacrophage serum is thus useful. A brief summary of its characteristics is thus presented.

III. Antilymphocyte Serum

The in vitro and in vivo lymphagglutinating and lymphocytotoxic properties of heterologous antilymphocyte sera have been long recognised. As early as 1899, Metchnikoff[51] injected spleen and lymph node cells of guinea pigs and rats into rabbits and observed both cytotoxic effects and agglutination of suspensions of such cells when later exposed to the sera of immunised rabbits. Excellent summaries of the chronology of the development of these sera have been presented by Gray et al.[52] and by Russell and Monaco, 1967.[53] However, it has only been in the last decade that growing awareness of the rôle of the lymphocyte in allograft rejection and the recognition of the need for immunosuppressive agents with fewer toxic side effects than standard immunosuppressives, has prompted the recent interest in specific antisera as immunosuppressive agents.

It was Woodruff in 1960[54] who first investigated the effects of antilymphoid sera on the survival of skin allografts in the rat. However, he was unable to demonstrate any significant prolongation. Subsequent investigations by Inderbitzin[55] revealed that antilymphocytic serum could suppress the cell mediated tuberculin reaction and his observations were later confirmed by Waksman et al.[56] Woodruff and Anderson[57,58] later demonstrated that antilymphocyte serum could significantly delay the rejection of first set skin allografts in rats across marked histocompatibility barriers.

Preparation and Purification

The preparation and purification of antilymphocyte serum has been reviewed by James.[59] Investigators have differed considerably in their source of antigen, immunisation schedule, and the use of adjuvant. Among the sources of antigen used are thymocytes, subcellular fractions of thymocytes, lymph node cells, subcellular fractions of lymph node cells, peripheral blood lymphocytes, thoracic duct lymphocytes, and spleen cells.

Briefly, the antigen is injected into a suitable animal with or without Freund's adjuvant and the animal is bled after acceptable titres of antilymphocyte serum have been produced. The serum obtained is inactivated and then absorbed on erythrocytes or cellular stroma, and after sterilisation by Seitz filtration is stored at -20°C. The species in which serum has been produced has also varied. The rabbit and horse have been the most widely used animals, but cows and sheep

have been utilised by some investigators. James[59] and Pichlmayr[60] have both suggested that antisera raised in horses and rabbits are more effective than those raised by similar procedures in cows and sheep.

Antilymphocyte sera usually contain antibodies against antigens other than lymphocytes and other leukocytes. High titres of erythrocyte agglutinins and lysins may be produced even when one uses pure preparations of antigen. In addition, antibodies against serum proteins are frequently detected. Thus, the serum is first inactivated by incubating at 56°C for 30 minutes. This results in the precipitation of an appreciable amount of fibrinogen which is removed by centrifugation. The serum is then absorbed with washed homologous erythrocytes. Iwasaki et al.[61] frequently absorb their antisera with inactivated whole serum of the lymphoid antigen donor species. Soluble antigen-antibody complexes are then removed by centrifugation.

Antilymphocyte Globulin

Observations to date have shown that the bulk of antilymphocyte activity of a number of sera has been located in the IgG fractions.[62,63] Iwasaki et al.[61] routinely employ the technique of ammonium sulphate precipitation to prepare this fraction. Woodruff et al.[62] using the technique of gel filtration on G200 sephadex have found that the major part of the lymphoagglutinating, lymphocytotoxicity and lymphocyte transforming activity is located in the 7S region whereas the bulk of the erythrocyte agglutinating activity is found in the 19S region. Furthermore, the 7S fraction proved as effective as the original anti-

serum.

The advantages of using purified antibody preparations has been stressed.[64] It avoids administering large amounts of extraneous nonantibody, potentially toxic protein. In addition, 'purified' antibody is concentrated and large amounts can thus be administered in small volume. Finally, by means of fractionation, the antilymphocyte activity can be resolved from the bulk of the erythroagglutinating activity, thus reducing the need for extensive adsorption.[62]

In Vitro Effects

All of the reports on the in vitro effects of antilymphocyte serum to date have demonstrated that antilymphocyte antibody combines with lymphocytes and causes their agglutination. In the presence of complement, the lymphocytes are lysed. The specific uptake of antilymphocyte antibody has been demonstrated by immunofluorescence.[53] Furthermore, in the absence of complement, combination of the antibody with lymphocytes causes lymphocyte transformation.

Antilymphocyte sera have been shown to be essentially species specific, but not strain specific.[52,65] In addition, cross reactivity with a variety of cells has been reported. These cells include erythrocytes, other lymphoid cells, platelets,[66] mast cells,[67] hemopoetic stem cells. Woodruff has demonstrated that antilymphocyte sera do not cause marked lysis of peritoneal macrophages.[65]

In Vivo Effects

Injection of antilymphocyte serum results in a transient

lymphopenia. This is more profound after repeated injections. However, even with prolonged courses of treatment, blood lymphocyte levels show signs of recovery during the course of treatment.[59] Antithymocyte sera[68] and sera produced with the aid of adjuvants[52] produce more effective lymphopenia.

Other blood cells may be affected, some authors reporting transient effects on granulocytes, eosinophils, monocytes and thrombocytes.[61]

The reported in vivo effects of antilymphocyte serum on lymphoid organs has been somewhat inconsistent. The histological changes in lymphoid tissues reported have varied from hypertrophy[69] to marked depletion.[52,70] Monaco et al.[71] have reported profound depletion in animals thymectomised prior to starting antilymphocyte serum administration. The existing literature on these changes has been summarised by James.[59]

Effects of Antilymphocyte Serum on Prolongation of Allograft Survival

The immunosuppressive properties of antilymphocyte serum have now been well documented and it has been employed by several investigators as an immunosuppressive agent both in experimental animals and in humans.

Investigators have differed considerably in a number of factors:

1) source of antigen used in the development of the antiserum,

2) route and schedule of immunisation of the animal,

3) in vitro potency of the serum obtained, and

4) route and regimen of antilymphocyte serum administration.

However, prolonged graft survival has been reported in almost all cases. The results of Monaco et al.[70] suggest that the immunosuppressive effect is most marked if the antiserum is given prior to grafting and can be further increased by continuing the treatment after grafting. This is in agreement with the results of Clunie et al.[72] and Starzl et al.[73] Pichlmayr,[60] on the other hand, has successfully prolonged allograft survival by starting antilymphocyte serum therapy on the day before or the day of grafting. Levey and Medawar[74] found that antilymphocyte serum was effective if started after grafting.

Other Effects of Antilymphocyte Serum

Other cellular phenomena.-- Levey and Medawar[74] have demonstrated that rabbit antisera to guinea pig lymph node cells abolished all components of the normal and sensitised lymphocyte transfer reactions.

It has also been shown that antilymphocyte serum will suppress the graft versus host reaction[75] and in conjunction with thymectomy has permitted the establishment of specific immunological tolerance.[76]

The suppression of delayed hypersensitivity to tuberculin in guinea pigs has been reported by several investigators.[56]

Humoral immune responses.-- Antilymphocyte sera suppress or delay primary humoral antibody responses. This has been studied in a number of systems, a summary of which has been presented by James.[59]

Complications of Antilymphocyte Therapy

Antilymphocyte serum, administered by the intravenous or intraperitoneal route, has frequently resulted in toxic manifestations especially if partially adsorbed or unadsorbed serum is used. Massive hemolysis, anemia and bloody diarrhea have been reported. In contrast, other investigators have found that extensively adsorbed antilymphocyte sera can be administered safely by the intravenous route. Iwasaki et al.[61] have reported that the normally high incidence of histologic renal damage observed in animals receiving prolonged courses of antilymphocyte serum therapy is reduced by the administration of semi-purified material by the subcutaneous route. Other investigators have noted that the material is less toxic if administered by this route.

Other complications include erythema, tenderness and pruritis at the site of injection, low grade fever, tachycardia, rashes, and generalised pruritis. A troublesome side effect is the high incidence of thrombocytopenia.[59]

Mode of Action of Antilymphocyte Serum

The theories postulated in an attempt to explain the mode of action of antilymphocyte serum has been reviewed by James.[59] Briefly, these theories are:

1) the cytotoxic theory,

2) the blindfolding theory,

3) the competitive antigen theory, and

4) the sterile inactivation theory.

The best formulation at present is the following:[77]

> Antilymphocyte serum binds to lymphocytes that are most accessible, that is, those in peripheral circulation. These cells are removed either through direct complement-dependent lysis or through the liver or spleen. This results in diminished lymphocyte counts in the peripheral blood and thoracic duct. Short lived bone marrow lymphocytes are rapidly restored while the thymus dependent population may remain depleted for a long time. Thus humoral antibody responses are relatively preserved.

IV. Adjuvants

In the preparation of antisera, many investigators have combined their challenging antigen with Freund's adjuvant in order to produce more potent antisera. The effects of adjuvants are twofold:

1) they increase antibody production, and

2) they effect a 'switch mechanism' and determine whether animals will respond to administration of antigen by tolerance or immunity.[78]

Freund,[79] in 1956, showed that incomplete adjuvant (a mixture of paraffin oil and mannoside monooleate) increased and prolonged antibody production while incorporation of Mycobacterium butyricum into the incomplete adjuvant further increased antibody production and accentuated delayed hypersensitivity. He postulated that incomplete adjuvant heightened antibody production, disseminated some antigen throughout the body, and stimulated a mononuclear cell reaction. He believed that the paraffin oil extracted lipids from the Mycobacteria in the case of complete adjuvant, and these lipids were supposed to have a direct action on the function of phagocytic cells, thus stimulating the immunologic process.

It has been shown that water-in-oil emulsions of antigens do form antigen depots.[80] However, other investigators have demonstrated that the antibody response to soluble antigens, e.g., bovine serum albumin, can be augmented by adjuvants injected at times and places remote from the antigen injection.[81-82] Moreover, repeated injections of high or low doses of bovine serum albumin tend to induce tolerance rather than to enhance the antibody response.[84] Bovine serum albumin and some other antigens may be carried to immunocompetent cells by macrophages, suggesting the possibility that specific cells might be the targets for adjuvant. Some adjuvants may act by damaging cell membranes.[83,85] Spitznagel and Allison[86] have demonstrated that several agents known to labilise lysosomal membranes have marked adjuvant effects.

Although it has been shown by Jeejeebhoy[87] and others that there is no direct correlation between the agglutination and cytotoxic titres and the immunosuppressive potency of any given antiserum, such titres, usually present at a significant level in most potent antisera, are still used as a crude criterion. It is therefore desirable to produce an antiserum with a high lymphoagglutinating and cytotoxic titre, and adjuvant assists in achieving this.

V. The Macrophage

Its Origin and Rôle in Rejection

There is now abundant evidence that the primitive precursors of macrophage stem cells are located mainly in the bone marrow. The blood borne cells giving rise to exudate macrophages are most likely to be

monocytes. Many workers have propounded the view that blood borne lymphocytes may transform into macrophages. However, there is considerable evidence against the lymphocyte being the major source of exudate macrophages. Excellent summaries of the evidence for and against these views has been presented by Nelson.[88]

The macrophage may play a part both in the afferent and efferent limbs of the immune response. Its rôle in the afferent limb consists of phagocytosis of the antigen and transfer of immunogenetic information to immunocompetent cells.[89-93] The interaction between macrophages and lymphocytes has already been discussed. Antigen is retained in macrophages for considerable periods of time and its immunogenicity is maintained. This may play a part in "immunological memory".[41]

The experiments of Granger and Weiner,[94,95] and Persall and Weiser,[96] have demonstrated that the macrophage may be effective in the efferent phase of the immunologic reflex and effect target cell destruction.

Argyris and Askonas[97] have demonstrated that the major part of antigen uptake by peritoneal cells is associated with large macrophages, whereas immunocompetence, as measured by the transfer of immunity to irradiated recipients, appeared to be due to small lymphocytes.

Properties and Some Uses of Antimacrophage Antibodies

Crude antisera to macrophages were prepared as early as 1899 by Metchnikoff but up to the present time have not been well characterised and they have found only limited use. Unanue,[98] using rabbits,

has developed a method of raising highly specific antisera to mouse macrophages, which show very poor or absent cross reaction with lymphocytes. He obtained peritoneal exudates from mice three to four days after intraperitoneal injection of 1.5 ml. of proteose peptone. Lymphocytes were eliminated by culturing the exudates for four days in Jena dishes with tissue culture medium "199" containing ten percent pooled rabbit sera. The latter sera were obtained from the rabbits to be immunised with the macrophages. After four days, the dishes were shaken hard and the floating dead cells and those which did not adhere to the glass were eliminated. These latter adhesive cells consisted almost exclusively of macrophages. They were detached from the glass with a rubber policeman, washed several times with cold tissue culture medium and suspended in saline containing merthiolate or incorporated in Freund's adjuvant. Rabbits were immunised twice at two week intervals, with a total of six million macrophages in 10 ml. of Freund's adjuvant and bled four weeks after the last injection. An alternative immunisation schedule used consisted of nine intravenous injections over a period of four weeks. These rabbits were bled eight days after the final injection.

He found that his antimacrophage sera agglutinated mouse peritoneal macrophages but not lymphocytes or mast cells. They also exerted cytotoxic effects on macrophages in the presence of complement, as shown by Trypan blue staining. There was marked inhibition of uptake of red cells or hemocyanin by live macrophages treated with

antimacrophage serum, probably as a consequence of membrane alteration by the antibody. Live macrophages treated with antimacrophage serum and allowed to settle on a petri dish, lost their ability to adhere to glass.

Unanue performed in vivo studies on antimacrophage serum, and found that if injected intraperitoneally into mice, it caused drastic changes in the cellular content of the peritoneal exudate. Macrophages were agglutinated in large masses, many of which contained numerous cell ghosts and the clusters of cells were surrounded by polymorphs. Lymphocytes, in general, were free in the exudate. Macrophages which had not been killed showed marked cellular aberrations and nuclear malformations. Intravenous injection of antimacrophage serum in large doses (0.5 ml.) led to death of mice. At autopsy there was wide-spread congestion of organs. Smaller doses (0.3 - 0.4 ml.) induced marked changes in the red pulp of the spleen including diminished numbers of cells in the sinuses which showed dilatation and hemorrhage. There were no changes in the white pulp. No impairment in the antibody response to foreign antigen (keyhole limpet hemocyanin) was observed. Dyminski and Argyris,[99] using antimacrophage serum as an immunosuppressive, have reported a prolongation of skin allograft survival comparable to that obtained with antilymphocyte serum.

Antimacrophage serum inhibits ability of specific antigens to induce blast transformation of previously sensitised circulating lymphocytes, without altering their response to nonspecific mitogens.[100]

VI. Intestinal Allotransplantation

In contrast to the volume of literature published on the fate of allografts of skin and kidney, there has been relatively little work done on intestinal allografts.

Allografts of the intestine have been established in dogs by Lillehei and his associates,[101-104] who observed that segments of jejunum transplanted to the recipient's neck underwent rejection in six to nine days in animals in which no attempt was made to control rejection with immunosuppressives. These investigators described a technique for the orthotopic transplantation of the entire small intestine, and found that the isolated small intestine could withstand periods of anoxia, effected by clamping of the superior mesenteric vessels, for as long as three to four hours with return of normal function when the obstruction was relieved. They also found that following autotransplantation of the entire small intestine lymphatic regeneration, as evidenced by the crossing over of the mesenteric suture line of sky blue dye, injected into the subserosa of the small intestine or into the mesenteric node of the autograft, occurred within two weeks.

Preston et al.[105] transplanted segments of small intestine into the necks of recipient dogs. They found that thrombosis at the sites of vessel anastomoses was a troublesome problem and this accounted for failure in 22 of 37 animals. They reported that rejection occurred at a mean of eight days in dogs untreated with immunosuppressive drugs. The use of the immunosuppressives, azathioprine and prednisone, resulted in

a significant prolongation in the survival of the allografts. With a combination of these two drugs, rejection occurred at a mean of 39 days. Analysis of the secretion from the grafted segment of small intestine showed concentrations of sodium, chloride, bicarbonate and potassium, similar to those in secretions of isolated intestinal fistulas. These investigators also described the gross and microscopic changes occurring in the rejection of intestinal allografts. Grossly, there was cyanosis and loss of peristaltic activity. Microscopically, round cell infiltration and hemorrhage into the mucous membrane was followed by sloughing of the superficial layers of the epithelium. Later, edema and perivascular round cell infiltration of the muscular and serosal layers occurred. The mucosa underwent destruction and replacement with a hemorrhagic exudate, the muscular layers remaining viable longer than the mucous membrane. In the later stages, infiltration by neutrophils was plentiful.

State[106] carried this series of experiments one stage further. In addition to the use of azathioprine and prednisone, he employed prolonged thoracic duct drainage and tested the absorptive function of the grafted bowel. He found that microscopically the earliest signs of rejection occurred at a mean of 5.6 days, and complete histological rejection was seen at seven days in dogs which were not given immunosuppressives. Prolonged thoracic duct drainage produced no prolongation of survival and combinations of azathioprine and adrenal steroids did not bring about consistent prolonged survival. In one dog there was apparent survival of the graft to 30 days; however, histologically, the

mucosa was no longer present and the muscular coats were replaced by fibrous tissue. He found that the functional capacity of the graft could be determined by differential absorption patterns of intraluminally instilled ^{14}C-d-arabinose and ^{14}C-d-glucose. Conversion of an active to a passive mode of absorption of ^{14}C-d-glucose characterised rejection and appeared well before gross evidence of rejection could be seen.

FOOTNOTES

[1]MEDAWAR, P.B. The behavior and fate of skin autografts and skin homografts in rabbits. J. Anat., 78:176, 1944.

[2]PERSALL, N., and WEISER, R. The macrophage in allograft immunity 1. Effects of silica as a specific macrophage toxin. J. Reticuloendothelial Soc., 5:107-120, 1968.

[3]LOEB, L. The biological basis of individuality. Springfield Thomas, 1945.

[4]MITCHISON, N.A. Passive transfer of transplantation immunity. Nature (Lond.), 171:267, 1953.

[5]BILLINGHAM, R.E., BRENT, L., MEDAWAR, P.B., and SPARROW, E.M. Quantitative studies on tissue transplantation immunity. 1. The survival times of skin homografts exchanged between members of different inbred strains of mice. Proc. Roy. Soc. Biol., 143:603, 1954.

[6]WAKSMAN, B.H. The pattern of rejection in rat skin homografts and its relation to the vascular network. Lab. Invest., 12:46, 1963.

[7]WEINER, J., SPIRO, D., and RUSSELL, P. An electron microscopic study of the homograft reaction. Am. J. Pathol., 44:319, 1964.

[8]BILLINGHAM, R.E. Lymphocyte response to antigens. B. Efferent side of sensitisation arc: Evidence for cytopathic effects of lymphocytes. Transplantation, 5:976, 1967.

[9]GOWANS, J.L., and McGREGGOR, D.J. The immunological activities of lymphocytes. Prog. Allergy, 9:1, 1965.

[10]GOWANS, J.L., McGREGGOR, D.J., and COWEN, D.M. The role of small lymphocytes in the rejection of homografts of skin, The Immunologically Competent Cell: Its Nature and Origin, (Ciba Foundation Study Group No. 16), ed. by G.E.W. Wolstenholme and J. Knight (Little, Brown and Company, Boston, 1963), p. 20-37.

[11]McGREGGOR, D.J. Bone marrow origin of immunologically competent lymphocytes in the rat. J. Exp. Med., 125:953, 1968.

[12]MILLER, J.F.A.P., and MITCHELL, G.F. Transplantation Rev., 1:1, 1969.

[13]ASPINALL, R., and MEYER, R. Effect of steroidal and surgical bursectomy and surgical thymectomy on the skin homograft reaction in chickens, Thymus in Immunobiology, ed. by R.A. Good and Ann Gabrielson (Hoeber [Harper Row], 1963), p. 376-394.

[14]MILLER, J.F.A.P. Immunological significance of the thymus, Advances in Immunology, vol. 2, ed. by W. Taliaferro and J.H. Humphrey (Academic Press, New York and London, 1962).

[15]MILLER, J.F.A.P. The thymus and transplantation immunity. Brit. Med. Bull., 21:111, 1965.

[16]ARNASON, G.B., JANKOVIC, B.D., and WAKSMAN, B.H. Role of the thymus in immune reactions in rats, Thymus in Immunobiology, ed. by R.A. Good and Ann Gabrielson (Hoeber [Harper Row], 1962), p. 492-500.

[17]MARTINEZ, C., DALMASSO, A.P., and GOOD, R.A. Homotransplantation of normal and neoplastic tissue in thymectomised mice, Thymus in Immunobiology, ed. by R.A. Good and Ann Gabrielson (Hoeber [Harper Row], 1962), p. 465-477.

[18]WAKSMAN, B.H., ARNASON, B.G., and JANKOVIC, B.D. Changes in the lymphoid organs of rats thymectomised at birth. Federation Proc., 21(Part 2):274, 1962.

[19]COOPER, M.D., PETERSON, R.D.A., SOUTH, M.A., and GOOD, R.A. Functions of the thymus system and bursa system in the chicken. J. Exp. Med., 123:75, 1966.

[20]MARCHESI, V.T., and GOWANS, J.L. The migration of lymphocytes through the endothelium of venules in lymph nodes: An electron microscopic study. Proc. Roy. Soc. (Biol.), 159:283, 1964.

[21]DAVIES, A.J.S. Transplantation Rev., 1:43, 1969.

[22]WISSLER, R., FITCH, F., and LAVIN, M. The relations of the R.E.S. to infection and immune reactions. The R.E.S. in antibody formation. Ann. N.Y. Acad. Sci., 88:134, 1960.

[23]THORABECKE, G.J., and BENACERRAF, B. The reticulo-endothelial system and immunological phenomena. Prog. Allergy, 6:559, 1962.

[24]EHRLICH, W.E. Lymphoid tissues: Their morphology and role in the immune response, Immunological Diseases, ed. by Samter, Little Brown Co., (Boston, 1965), p. 251-277.

[25]FITCH, F.W., and WISSLER, R.W. The histology of antibody production, Immunological Diseases, ed. by Samter, Little Brown Co., (Boston, 1965), p. 65-86.

[26]DePETRIS, S., and KARLSBAND, G. Localization of antibodies by electron microscopy in developing antibody-producing cells. J. Cell Biol., 26:759, 1965.

[27]HARRIS, T.N., HUMMELER, K., and HARRIS, S. Electron microscopic observations on antibody-producing lymph node cells. J. Exp. Med., 123:161-172, 1966.

[28]GREAVES, M.F., TORRIGIANI, G., and ROITT, I.M. Blocking of the lymphocyte receptor site for cell mediated hypersensitivity and transplantation reactions by anti-light chain sera. Nature (Lond.), 222:885, 1969.

[29]SELL, S. Studies on rabbit lymphocytes in vitro v. the induction of blast transformation with sheep antisera to rabbit IgG subunits. J. Exp. Med., 125:289, 1967.

[30]SELL, S., and GELL, P.G.H. Studies on rabbit lymphocytes in vitro. I. Stimulation of blast transformation with anti-allotype serum. J. Exp. Med., 122:423, 1965.

[31]PIERCE, C.W. Immune responses in vitro. I. Cellular requirements for the immune response by nonprimed and primed spleen cells in vitro. J. Exp. Med., 130:345-363, 1969.

[32]FISHMAN, M. Antibody formation in vitro. J. Exp. Med., 114:837, 1961.

[33]MOISER, D.E. Cell interactions in the primary response in vitro: a requirement for specific cell clusters. J. Exp. Med., 129:351, 1969.

[34]MOISER, D.E. A requirement for two cell types for antibody formation in vitro. Science (Washington), 158:1575, 1967.

[35]MOISER, D.E., and COPPLESON, L.W. A three cell interaction required for the induction of a primary immune response in vitro. Proc. Nat. Acad. Sci. (USA), 61:542, 1968.

[36]GALLILY, R., and FELDMAN, M. The role of macrophages in the induction of antibody in X-irradiated animals. Immunology, 12:197, 1967.

[37]FISHMAN, M., and ADLER, F.L. The role of macrophage RNA in the immune response. Cold Spring Harbor Symp. Quant. Biol., 32:343, 1968.

[38]SCHONENBERG, M.D., MUMOW, V.R., MOORE, R.D., and WEISBERGER, A.S. Cytoplasmic interaction between macrophage and lymphocytic cells in antibody synthesis. Science (Washington), 143:964, 1967.

[39]ARGYRIS, B.F. Role of macrophages in antibody production. Immune response to sheep red blood cells. J. Immunol., 99:774, 1967.

[40]ARGYRIS, B.F. Role of macrophages in immunological maturation. J. Exp. Med., 127:459, 1968.

[41]UNANUE, E.R., and ASKONAS, B.A. Persistence of immunogenicity of antigen after uptake by macrophages. J. Exp. Med., 127:915, 1968.

[42]HERSH, E.M., and HARRIS, J.E. Macrophage-lymphocyte interaction in the antigen-induced blastogenic response of human peripheral blood leukocytes. J. Immunol., 100:1184, 1968.

[43]CLINE, M.J., and SWETT, V.C. The interaction of human monocytes and lymphocytes. J. Exp. Med., 128:1309, 1968.

[44]KOLSCH, E., and MITCHISON, N.A. The subcellular distribution of antigen in macrophages. J. Exp. Med., 128:1059, 1968.

[45]UNANUE, E.R., and ASKONAS, B.A. Two functions of macrophages and their role in immune responses. J. Reticulo-endothelial Soc., 4:440, 1967.

[46]HOLM, G., PERLMANN, P., and WERNER, B. Phytoaemagglutinin-induced cytotoxic action of normal lymphoid cells on cells in tissue culture. Nature (Lond.), 203:841, 1964.

[47]MÖLLER, E. Contact-induced cytotoxicity by lymphoid cells containing foreign isoantigens. Science, 147:873, 1965.

[48]GINSBERG, H. Graft versus host reaction in tissue culture. I. Lysis of monolayers of embryomouse cells from strains differing in the H-2 histocompatibility locus by rat lymphocytes sensitised in vitro. Immunology, 14:621, 1968.

[49]DAVID, J.R. Macrophage migration. Fed. Proc., 27:6, 1968.

[50]BLOOM, B.R., and BENNET, B. Migration inhibitory factor associated with delayed-type hypersensitivity. Fed. Proc., 27:13, 1968.

[51]METCHINKOFF, E. Ann. Inst. Past., 13:737, 1899.

[52]GRAY, J.G., MONACO, A.P., WOOD, M.L., and RUSSELL, P.S. Studies on heterologous anti-lymphocytic serum in mice. I. In vitro and in vivo properties. J. Immunol., 96:217-228, 1966.

[53]RUSSELL, P.S., and MONACO, A.P. Heterologous anti-lymphocyte sera and some of their effects. Transplantation, 5:1086-1099, 1967.

[54]WOODRUFF, M.F.A. The transplantation of tissues and organs, (Thomas, Springfield), Illinois, 1960.

[55]INDERBITZIN, T. The relationship of lymphocytes, delayed cutaneous allergic reactions and histamine. Int. Arch. Allergy, 8:150-159, 1956.

[56]WAKSMAN, H.B., ARBUOYS, S., and ARNASON, B.C. The use of specific lymphocyte anti-sera to inhibit hypersensitive reactions of the delayed type. J. Exp. Med., 114:997-1021, 1961.

[57]WOODRUFF, M.F.A., and ANDERSON, N.F. Effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocytic serum on the survival of skin homografts in rats. Nature (Lond.), 200:702, 1963.

[58]WOODRUFF, M.F.A., and ANDERSON, N.F. The effect of lymphocyte depletion by thoracic duct fistula and the administration of anti-lymphocytic serum on the survival of skin homografts in rats. Ann. N.Y. Acad. Sci., 120:119-128, 1964.

[59]JAMES, K. The preparation and properties of antilymphocytic sera. Progr. Surg., 7:140-216, 1969.

[60]PICHLMAYR, R., BRENDEL, W., and ZENKER, R. Production and effect of heterologous anti-canine lymphocyte serum. Surgery, 61:774, 1967.

[61]IWASAKI, Y., PORTER, K.A., AMEND, J.R., MARCHIORD, T.L., ZUHLKE, V., and STARZL, T.E. The preparation and testing of horse anti-dog and anti-human anti-lymphoid plasma or serum and its protein fractions. Surg. Gynec. Obstet., 124:1-24, 1967.

[62]WOODRUFF, M.F.A., JAMES, K., ANDERSON, N.F., and REID, B.L. Comparison of in vivo and in vitro properties of ALS; in Ciba Study Group on Anti-Lymphocyte Serum, p. 57-58, (Churchill, London, 1967a).

[63]JAMES, K., and MEDAWAR, P.B. Characterisation of antilymphocyte antibody. Nature (Lond.), 214:1052-1053, 1967.

[64]JAMES, K. Anti-lymphocytic antibody-A review. Clin. Exp. Immunol., 2:615-631, 1967.

[65]WOODRUFF, M.F.A., ANDERSON, N.F., and ABAZA, H.M. Experiments with antilymphocytic serum, Bristol Symposium on the Lymphocyte in Immunology and Haemopoisesis, ed. by Edward Arnold (London, 1966), p. 286.

[66]STARZL, T.E., PORTER, K.A., IWASAKI, Y., MARCHIORO, T.L., and KASHIWAGI, N. The use of heterologous antilymphocyte globulin in human renal homotransplantation, Antilymphocytic Serum, (Ciba Foundation Study Group No. 29), ed. by G.E.W. Wolstenholme and M. O'Connor (Little, Brown and Company, Boston, 1967), p. 4-40.

[67]GUTTMAN, R.D., CARPENTER, C.B., LINDQUIST, R.R., and MERRILL, J.P. Renal transplantation in the inbred rat. III. A study of heterologous and antithymocyte sera. J. Exp. Med. 126:1099-1127, 1967.

[68]NAGAYA, H., and SIEKER, H.O. Allograft survival: Effect of antiserums to thymus glands and lymphocytes. Science, 150:1181-1182, 1965.

[69]LEVEY, R.H., and MEDAWAR, P.B. Nature and mode of antilymphocytic serum. Proc. Nat. Acad. Sci. (USA), 56:1130-1137, 1966.

[70]MONACO, A.P., ABBOTT, W.N., OTHERSON, H.B., SIMMONS, R.L., WOOD, M.L., FLAX, M.H., and RUSSELL, P.S. Antiserum to lymphocytes: Prolonged survival of canine renal allografts. Science, 153:1264-1267, 1966.

[71]MONACO, A.P., WOOD, M.L., and RUSSELL, P.S. Adult thymectomy: effect on recovery from immunologic depression in mice. Science, 149:432-435, 1965.

[72]CLUNIE, G.J.A., NOLAN, B., JAMES, K., WATT, J.G., and WOODRUFF, M.F.A. Prolongation of canine renal allografts with antilymphocytic serum. Transplantation, 6:459-467, 1968.

[73]STARZL, T.E., MARCHIORO, T.L., PORTER, K.A., IWASAKI, Y., and CERRILLI, C.J. The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal transplantation. Surg. Gynec. Obstet., 124:301-318, 1967.

[74]LEVEY, R.H., and MEDAWAR, P.B. Some experiments on the actions of anti-lymphoid antisera. Ann. N.Y. Acad. Sci., 129:164-177, 1966.

[75]BRENT, L., COURTENAY, T., and GOWLAND, G. Immunological reactivity of lymphoid cells after treatment with antilymphocytic serum. Nature (Lond.), 215:1461-1464, 1967.

[76]MONACO, A.P., WOOD, M.L., and RUSSELL, P.S. Studies on heterologous antilymphocyte serum in mice. III. Immunologic tolerance and chimerism produced across the H-2 locus with adult thymectomy and antilymphocyte serum. Ann. N.Y. Acad. Science Wash., 129:190-209, 1966.

[77]RUSSEL, P.J., and WINN, H.J. Transplantation (Part 2). New Eng. J. Med., 282:848, 1970.

[78]DRESSER, D.W. Effectiveness of lipid and lipidophilic substances as adjuvants. Nature, 191:1169, 1961.

[79]FREUND, J. Advances Tuberc. Res., 7:130, 1956.

[80]WEIGLE, W., DIXON, F.J., and DEICHMULLER, M.D. Effect of mycobacteria on antibody production induced by water in oil emulsions of soluble protein antigens. Proc. Soc. Exp. Biol. Med., 105:535, 1960.

[81]MERRITT, K., and JOHNSON, A.G. Studies on the adjuvant action of bacterial endotoxins on antibody formation. V. The influence of endotoxin and 5-Fluoro-2 Deoxyuridine on the primary antibody response of the BALB mouse to a purified protein antigen. J. Immunol., 91:266, 1963.

[82]GOLUB, E.S., and WEIGLE, W.O. Studies on the induction of immunologic unresponsiveness. 1. Effects of endotoxin and phytohemagglutinin. J. Immunol., 98:1241, 1967.

[83]DRESSER, D.W. Adjuvanticity of vitamin A. Nature, 217:527, 1968.

[84]MITCHISON, N.A. Induction of immunological paralysis in two zones of dosage. Proc. Roy. Soc. (Lond.), 161:275, 1965.

[85]GALL, D. The adjuvant activity of aliphatic nitrogenous bases. Immunology, 11:369, 1966.

[86]SPITZNAGEL, J.K., and ALLISON, A.C. Mode of action of adjuvants: Retinol and other lysosome labilising agents as adjuvants. J. Immunol., 104:119, 1970.

[87]JEEJEEBHOY, H.F. Studies on the mode of action of heterologous antilymphocyte plasma. I. A comparison of the immunosuppressive properties of dog and rabbit antirat lymphocyte plasma. Transplantation, 5:273, 1967.

[88]NELSON, D.S. Macrophages in Immunity, Frontiers in Biology Series, ed. by A. Neuberger and E.L. Tatum (North Holland Publishing Company, Amsterdam, London, 1969), p. 41.

[89]ASKONAS, B.A., and RHODES, J.M. Immunogenicity of antigen-containing ribonucleic acid preparations from macrophages. Nature, 205:470, 1965.

[90]FISHMAN, M., and ADLER, F.L. Antibody formation initiated in vitro. II. Antibody synthesis in X-irradiated recipients of diffusion chambers containing nucleic acid derived from macrophages incubated with antigen. J. Exp. Med., 117:595, 1963.

[91]FISHMAN, M., HAMMERSTROM, R.A., and BOND, V.P. In vitro transfer of macrophage RNA to lymph node cells. Nature (Lond.), 198:549, 1963.

[92]FRIEDMAN, A.P., STRAVISKI, A.B., and SOLOMON, J.M. Induction in vitro of antibodies to phage P-2: antigens in RNA extract employed. Science, 149:1106, 1965.

[93]GOTTLIEB, A.A. Antigens, RNAs, and macrophages. J. Reticuloendothelial Soc., 5:270, 1968.

[94]GRANGER, G.A., and WEISER, R.S. Homograft target cells: Specific destruction in vitro by contact interaction with immune macrophages. Science, 145:1427, 1964.

[95]GRANGER, G.A., and WEISER, R.S. Homograft target cells: Contact destruction in vitro by immune macrophages. Science, 151:97, 1966.

[96]PERSALL, N.N., and WEISER, R.S. The macrophage in allograft immunity. II. Passive transfer with immune macrophages. J. Reticuloendothelial Soc., 5:121, 1968.

[97]ARGYRIS, B.F., and ASKONAS, B. Mouse peritoneal cells: Their ability to elicit or produce antibody after exposure to antigen. Immunology, 14:379, 1968.

[98]UNANUE, E.R. Properties and some uses of antimacrophage antibodies. Nature, 218:36, 1968.

[99]DYMINSKI, J.W., and ARGYRIS, B.F. Prolongation of allograft survival with antimacrophage serum. Transplantation, 8:595, 1969.

[100]JEHN, U.W., MUSHER, D.M., and WEINSTEIN, L. The effect of antimacrophage serum on macrophage-lymphocyte interaction in vitro. Proc. Soc. Exp. Biol. Med., 134:241, 1970.

[101]LILLEHEI, R.C., GOOT, B., and MILLER, F.A. Physiological response of small bowel of the dog to ischemia including prolonged in vitro preservation of the bowel with successful replacement and survival. Ann. Surg., 105:543, 1959.

[102]GOOT, B., LILLEHEI, R.C., and MILLER, F.A. Homografts of small bowel. Surg. Forum, 10:193, 1959.

[103]LILLEHEI, R.C., GOLDBERG, S., GOOT, B., and LONGERBEAN, J.K. Present status of intestinal transplantation. Ann. J. Surg., 105:58, 1963.

[104]LILLEHEI, R.C., IDEZUKI, Y., FEEMSTER, J.A., DIETZMAN, R.H., KELLY, W.D., MERKEL, F.K., GOETZ, F.C., LYONS, A.W., and MANAX, W.G. Transplantation of stomach intestine and pancreas - experimental and clinical observations. Surgery, 62:721, 1967.

[105]PRESTON, F.W., MACALALAD, F., GRABER, R., JACKSON, E.J., and SPORN, J. Function and survival of jejunal homotransplants in dogs with and without immunosuppressive treatment. Transplantation, 3:224, 1965.

[106]STATE, D. Experimental studies in intestinal allografts. Surg. Clin. N. Amer., 49:547, 1969.

CHAPTER II

MATERIALS AND METHODS

I. Animals

Healthy, adult mongrel dogs of both sexes, weighing 16 - 26 kg. were used for all experiments. They were obtained from the University Farm.

Adult, common barnyard goats, obtained from a local supplier, were used for the preparation of antilymphocyte serum and antimacrophage serum. Seven female and two castrated male goats were used.

II. Preparation of Lymphocyte Suspension

Mesenteric lymph nodes were obtained from dogs that had been used in other acute experiments. These dogs had not been transfused or received antigen. The node tissue was carefully separation from all traces of fat and connective tissue, suspended in Ringer's solution, and manually homogenised. The resulting suspension was filtered twice through fine cheesecloth, washed in Ringer's solution, and the cells resuspended in Ringer's soltuion.

III. Stimulation and Collection of Peritoneal Cells[1]

Two hundred millitres of sterilised olive oil was introduced into the peritoneal cavity of a healthy dog. Seven days later laparotomy was performed and the peritoneal cavity washed with sterile

heparinised Hank's balanced salt solution.

IV. Preparation of Crude Macrophage Suspension

Peritoneal washings as obtained in the above were centrifuged at 2000 r.p.m. for 20 minutes. The cells obtained were washed thrice and subsequently resuspended in Hank's balanced salt solution. A slide made from this suspension was stained in Wright's stain and read microscopically. This method gave a cell suspension which contained approximately 70 percent macrophages and 30 percent lymphocytes.

V. Purification of Macrophages

Peritoneal washings as obtained above were incubated on columns of Ballotini glass beads for one hour at room temperature. The washings were allowed to escape at the bottom of the column. Peritoneal macrophages were washed off the column with 0.05 M disodium ethylene diamine tetra-acetate. The suspension was centrifuged at 2000 r.p.m. for 20 minutes and the macrophages subsequently resuspended in Hank's solution. A slide was made from this preparation, stained with Wright's stain and read microscopically. This method gave a macrophage suspension of 98 percent purity.

VI. Immunosuppressives

Preparation of Antisera

Antilymphocyte serum.-- Goat antidog lymphocyte serum was prepared in the following manner. Goats were given an initial intraperitoneal injection of 20 million lymphocytes in Freund's complete

adjuvant. Two weeks later a similar injection was given. After a further period of two weeks, the goats were bled via the external jugular vein. At monthly intervals, further booster injections were given and the goats were bled two weeks following each booster. Five hundred millilitres of blood was obtained at each bleeding.

Antimacrophage serum.-- Goat antidog macrophage serum was prepared in a similar manner to antilymphocyte serum. Goats were sensitised with 10 million purified macrophages (in the case of 'pure' antimacrophage serum) and 10 million peritoneal cells (crude macrophage suspension) in the case of 'impure' antimacrophage serum. Two weeks after the sensitising dose, goats were rechallenged with a similar dose of cells. They were bled two weeks after the booster injection.

Blood obtained from the goats were refrigerated overnight to allow it to clot. On the following morning, the clot was gently broken up and the serum removed by centrifugation.

Adsorption of Sera

Sera were decomplemented by heating to 56°C for 30 minutes. They were then adsorbed with dog erythrocytes in order to remove hemaglutinins. One volume of serum was incubated with two volumes of washed dog erythrocytes at 37°C for a half hour. The mixture was then centrifuged and the serum removed by pipette, placed in tubes and frozen until ready for use.

Testing of Antisera

Leukoagglutination.-- A modification of the method of Amos and Peacocke was used.[2] A lymph node cell suspension was prepared as described above and the cells were washed three times in 0.15 M NaCl. The cells were then diluted to a final concentration of 7×10^6/ml. in a buffer containing 2.6 g. $NaHPO_4$, 3.0 g. Na_2 EDTA, and 8.5 g. NaCl/litre. Equal volumes (0.05 ml.) of cells and serial dilutions of serum were mixed in 6 x 50 mm. siliconised tubes and incubated at room temperature for two hours. The suspensions were then mixed once by gentle agitation, dropped onto a glass slide, and read microscopically for agglutination as a wet preparation or, alternately, allowed to dry, stained with Wright's stain, and then read.

Cytotoxic test[2].-- Two drops of normal saline were placed in a shallow well formed by a Vaseline petroleum jelly ring on a glass slide. Dog spleen or lymph node tissue was cut cleanly across, and the cut surface repeatedly touched to the surface of the saline. Cells flowed freely into the fluid, and a suspension of 95 percent viability (Trypan blue staining) was regularly obtained. The cytotoxic activity of the goat antidog lymphocyte serum was determined by adding an equal volume of serum to the cell suspension and incubating for 30 minutes at 37°C. Following this, a drop of freshly prepared Trypan blue (0.20%) was added and microscopic counts of living (unstained) and dead (stained) cells were made.

VII. Preoperative Management of Dogs

Dogs were randomly chosen and each arbitrarily assigned to one of five groups as soon as it was received from the University Farm. Each group received a different regimen of immunosuppressive therapy.

Group I - Controls, received no immunosuppressive therapy.

Group II - Received antilymphocyte serum only in dosages of 5 ml. daily. Therapy was started three days preoperatively.

Group III - Received 'pure' antimacrophage serum only in a dosage of 5 ml. daily, starting three days preoperatively.

Group IV - Received a combination of antilymphocyte serum (5 ml. daily) and standard dosages of azathioprine* and prednisone¶. Antilymphocyte serum was started three days preoperatively and azathioprine and prednisone were given on the day after operation and daily thereafter. Starting doses of the two latter drugs were 200 mg. azathioprine and 25 mg. prednisone.

Group V - Received 'crude' antimacrophage serum only, in dosages of 5 ml. daily, starting three days preoperatively.

There was no attempt to match dogs except that, as far as possible, it was tried to have members of donor-recipient pairs of the same sex and roughly similar size for each operation.

All dogs were maintained on a standard laboratory diet until one

---

* azathioprine - Imuran from Burroughs Wellcome & Co., Canada Ltd.

¶ prednisone - Novopharm Ltd., Scarborough, Ontario.

day prior to operation and then fasted except for water.

Anaesthesia was achieved by the administration of intravenous sodium pentobarbital. The abdomen was shaved, scrubbed with Phisohex*, and painted with a solution of Betadine¶.

VIII. Operative Techniques (Figures 2-15)

Two animals were prepared simultaneously and segments of bowel exchanged, so that each animal was both a donor and recipient.

Animals were anaesthetised and prepared as described above. The abdomen was draped and entered through a vertical midline incision. The ileocecal junction was identified and the bowel divided 10 cm. proximal to it. About one metre of small intestine was measured proximally from this division and the bowel divided again. The mesentery was incised at these two points and the incisions extended to the root of the mesentery. The mesenteric nodes were divided transversely. The superior mesenteric vessels were then dissected free from all lymphatic tissue. This procedure resulted in an isolated segment of bowel pedicled on the superior mesenteric artery and vein.

An identical procedure was performed on the second dog. The mesenteric vessels were then clamped separately with bulldog clamps and divided distal to the clamps.

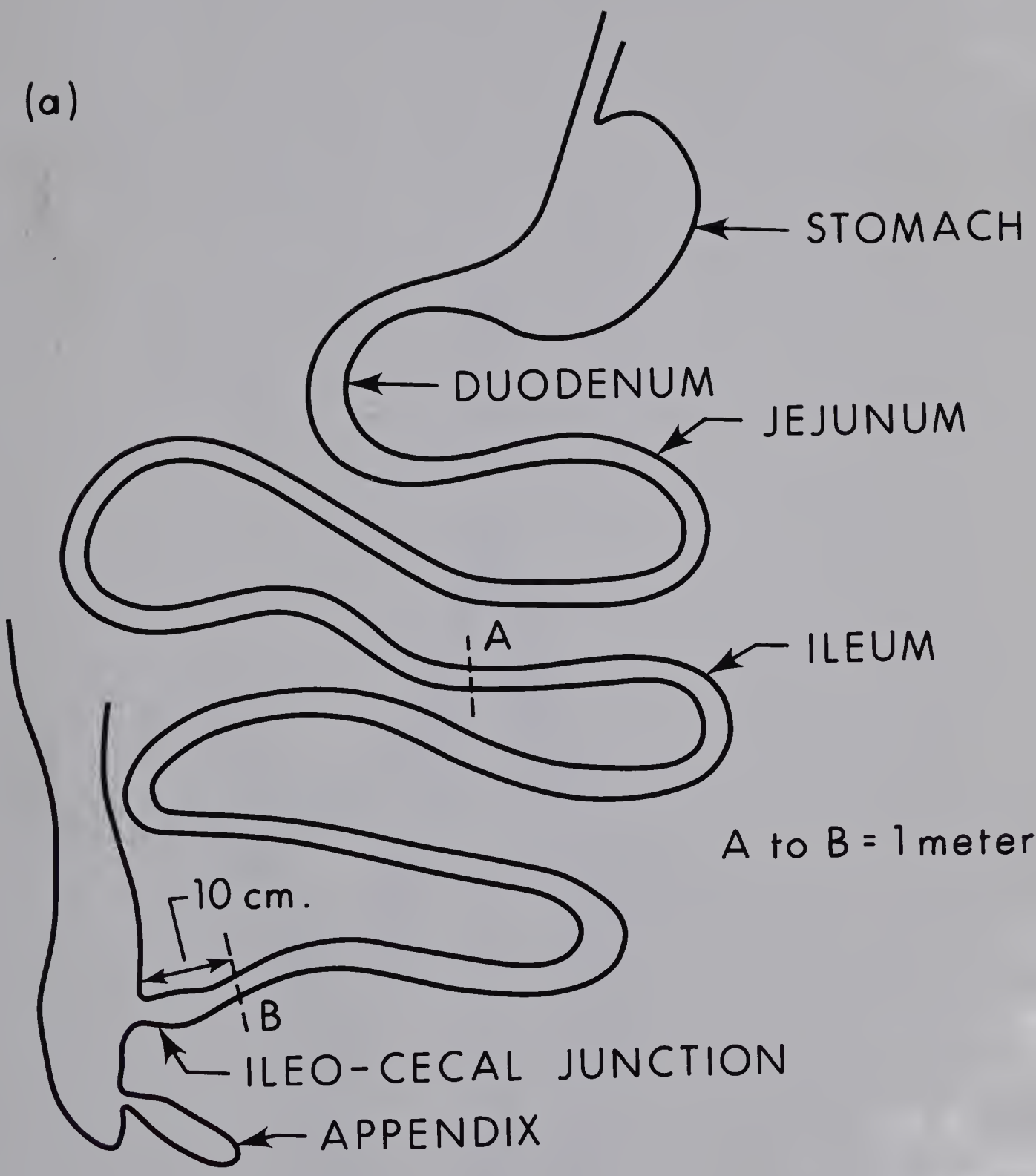
The excised segments of bowel were perfused with heparinised.

---

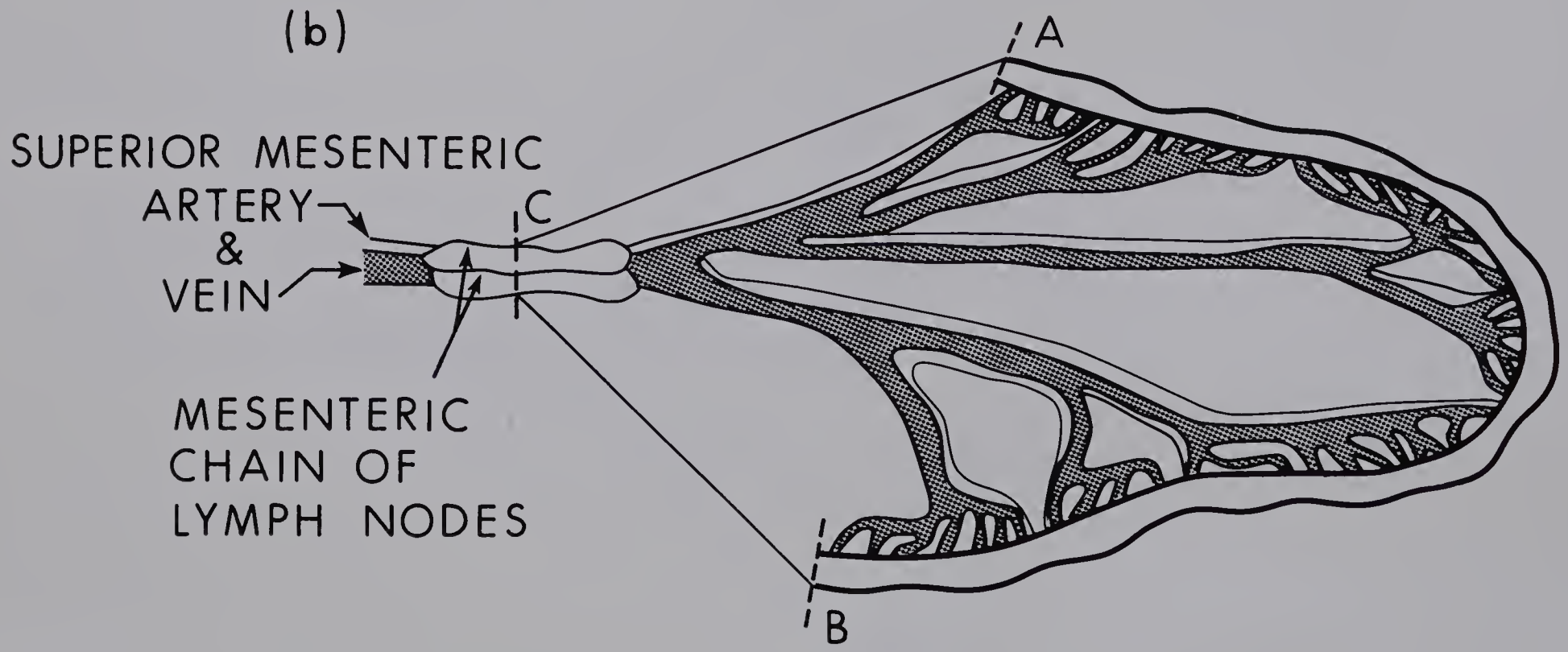
* Phisohex - Winthrop Laboratories, Canada Ltd., Aurora, Ontario.

¶ Betadine - Burroughs Wellcome, Canada Ltd., Montreal, Quebec.

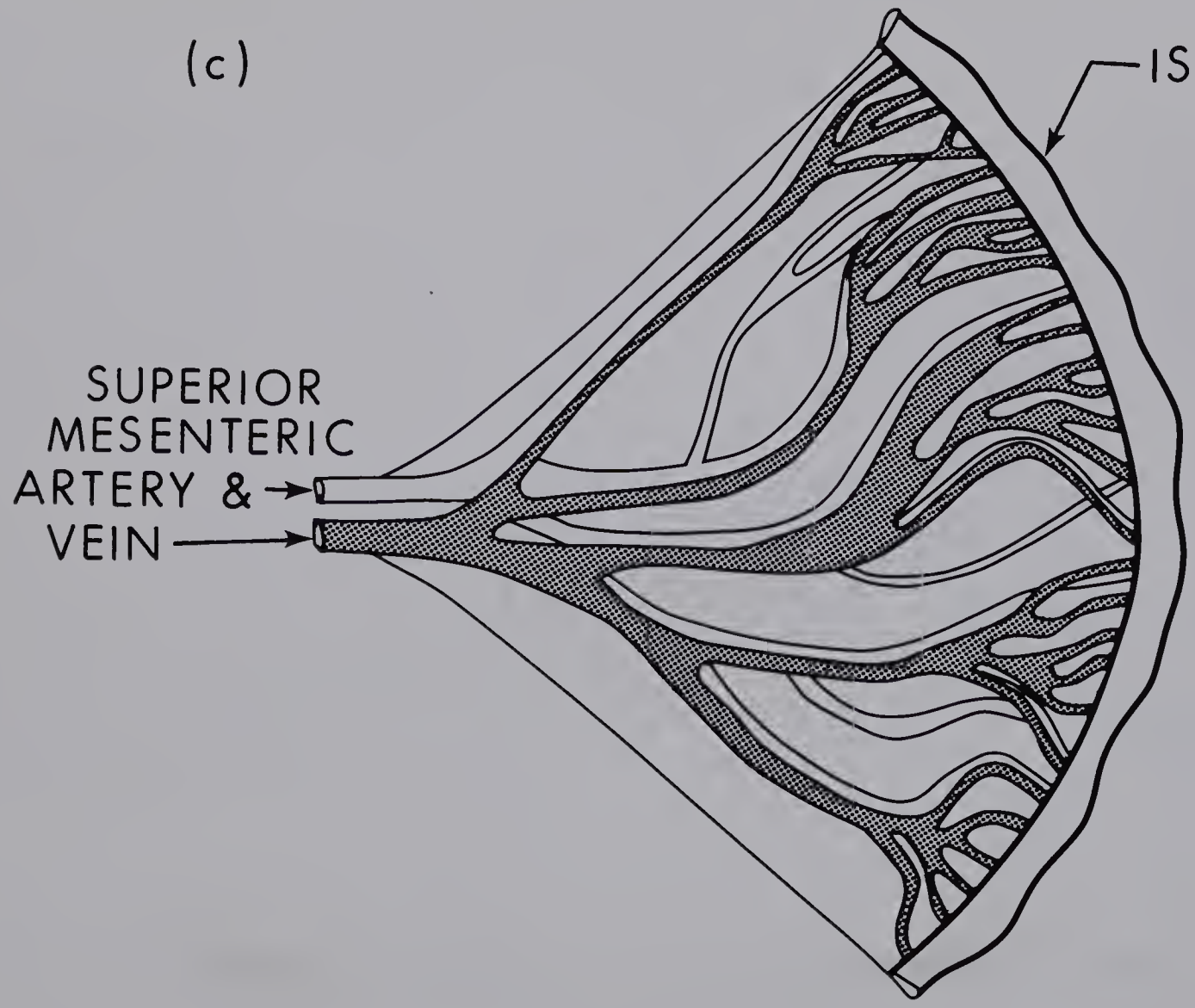
(a)


STOMACH
DUODENUM
JEJUNUM
A
ILEUM
A to B = 1 meter
10 cm.
B
ILEO-CECAL JUNCTION
APPENDIX

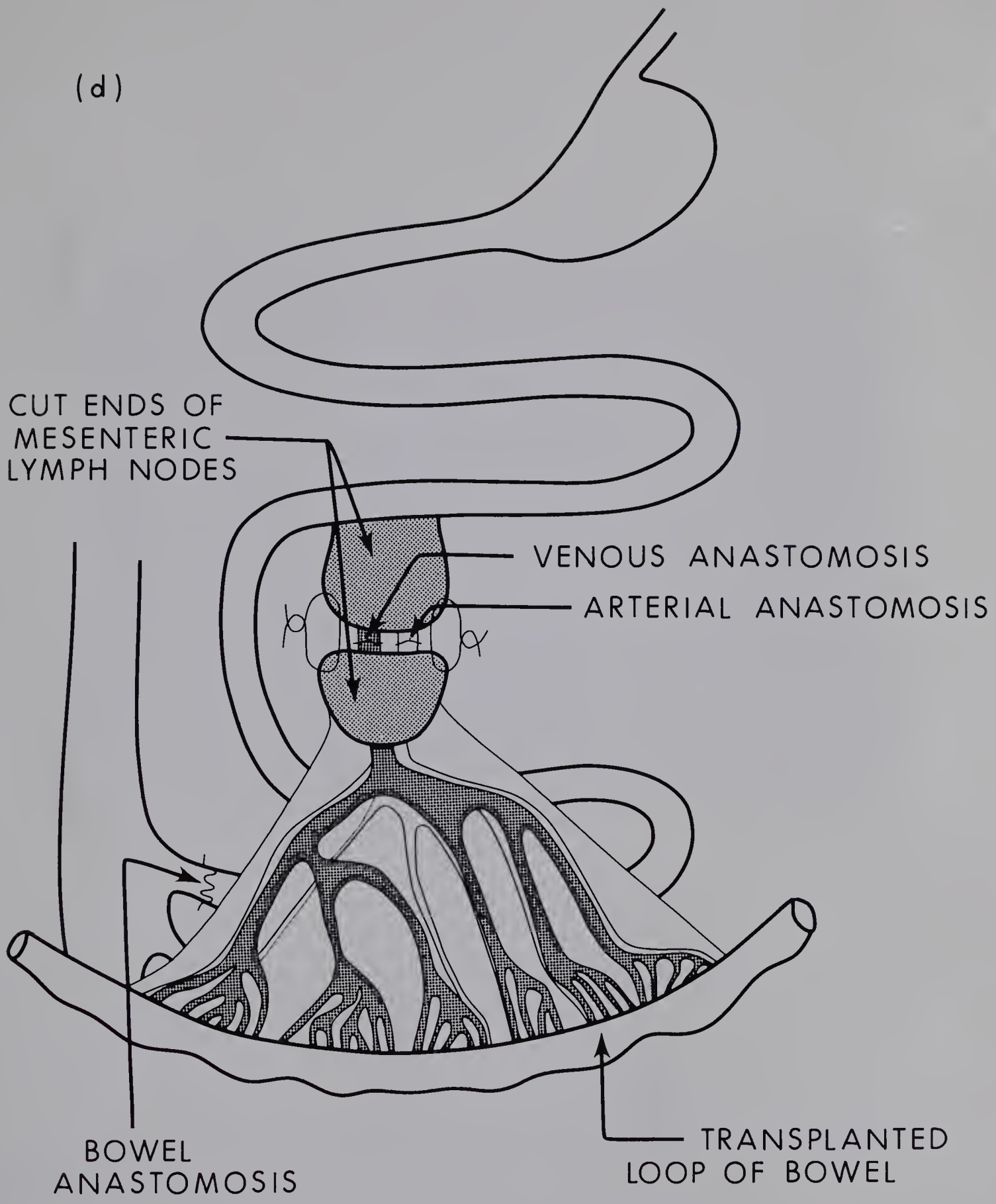
(b)


A
SUPERIOR MESENTERIC ARTERY & VEIN
C
MESENTERIC CHAIN OF LYMPH NODES
B

(c)



(d)


CUT ENDS OF MESENTERIC LYMPH NODES
VENOUS ANASTOMOSIS
ARTERIAL ANASTOMOSIS
BOWEL ANASTOMOSIS
TRANSPLANTED LOOP OF BOWEL

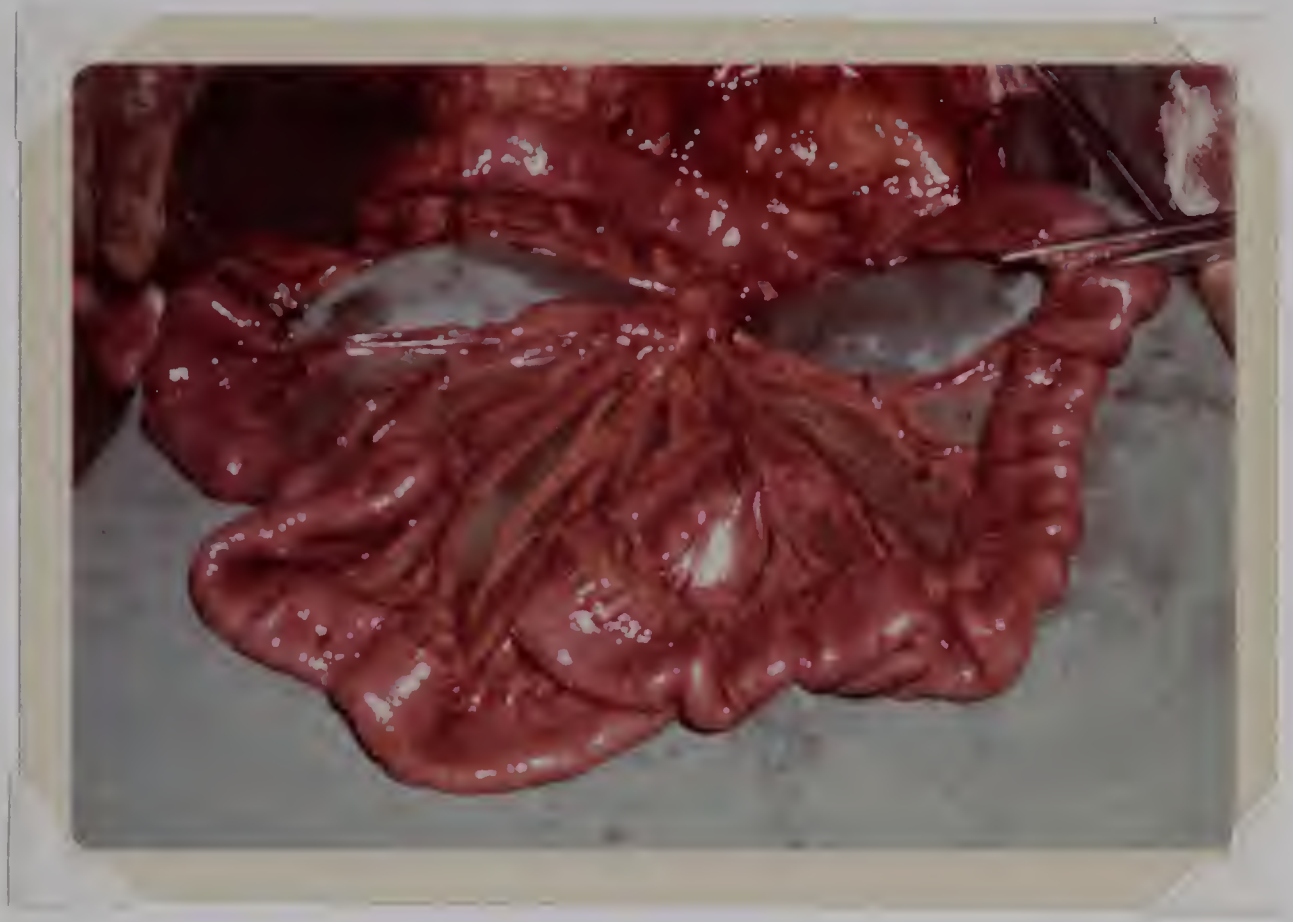


Fig. 6.-- Photograph showing points of division of small intestine.

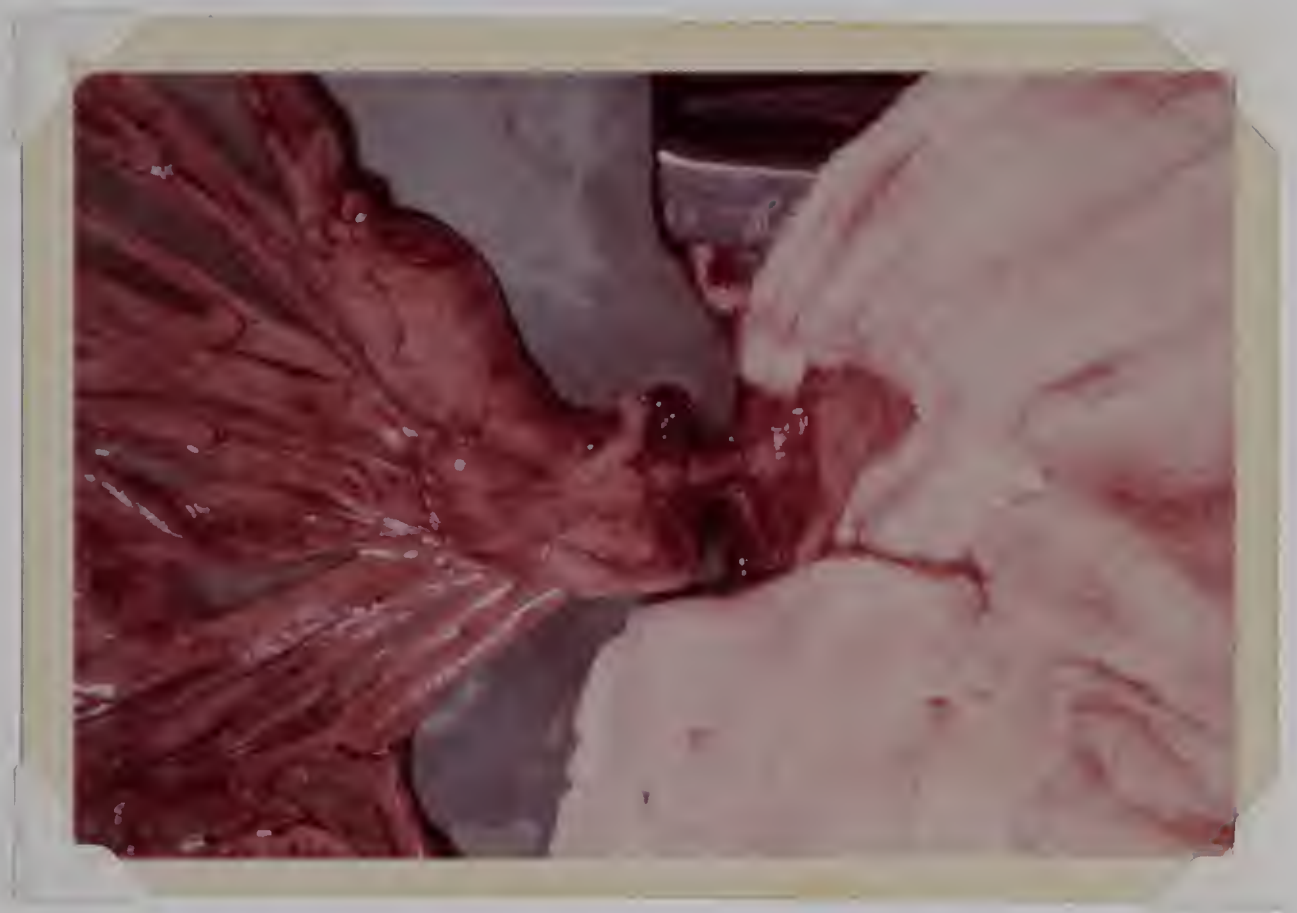


Fig. 7.-- Photograph after division of mesenteric lymph nodes showing vascular pedicle.

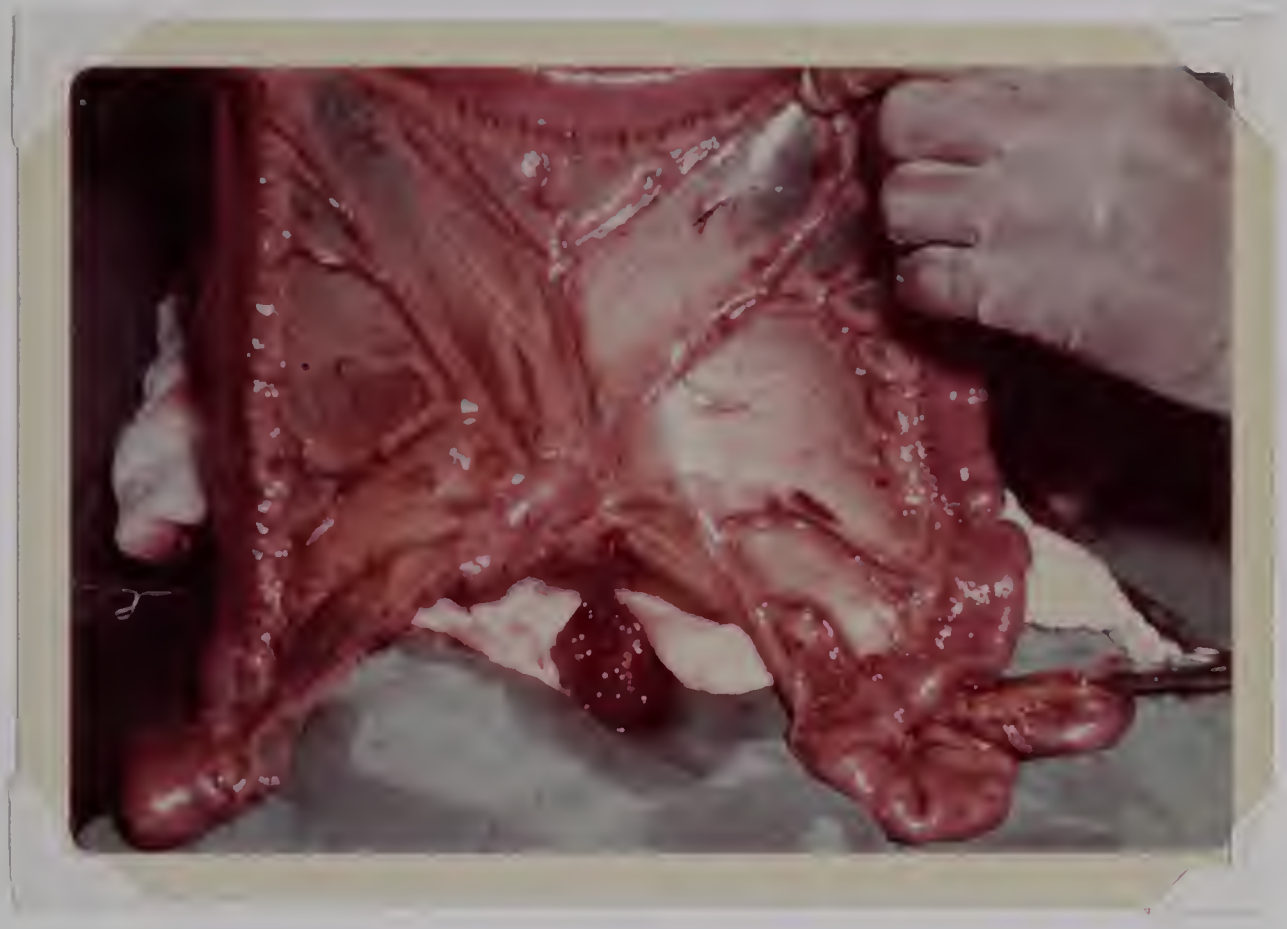


Fig. 8.-- Photograph showing loop of small intestine isolated on its vascular pedicle.

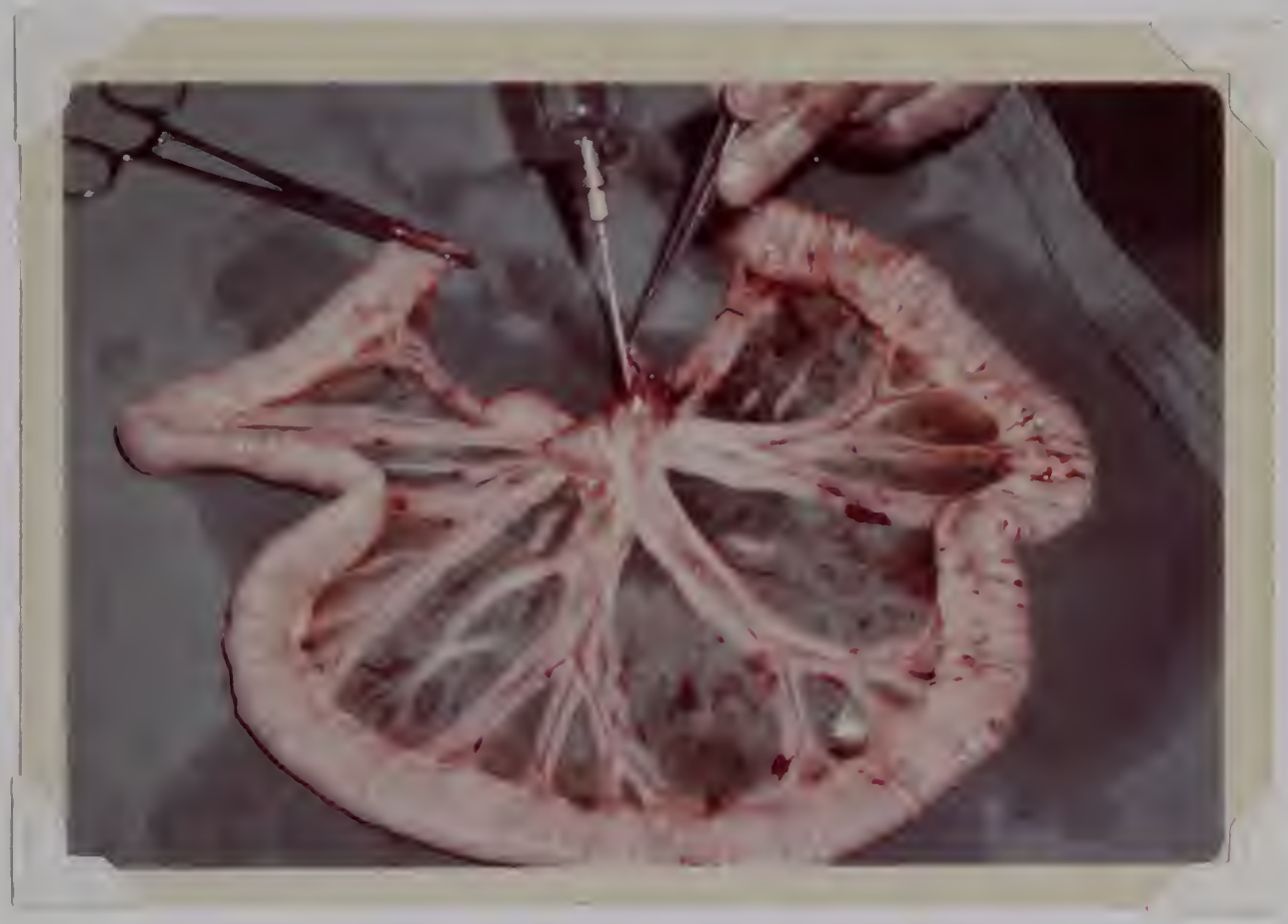


Fig. 9.-- Photograph showing perfusion of excised segment of small intestine via its main artery.

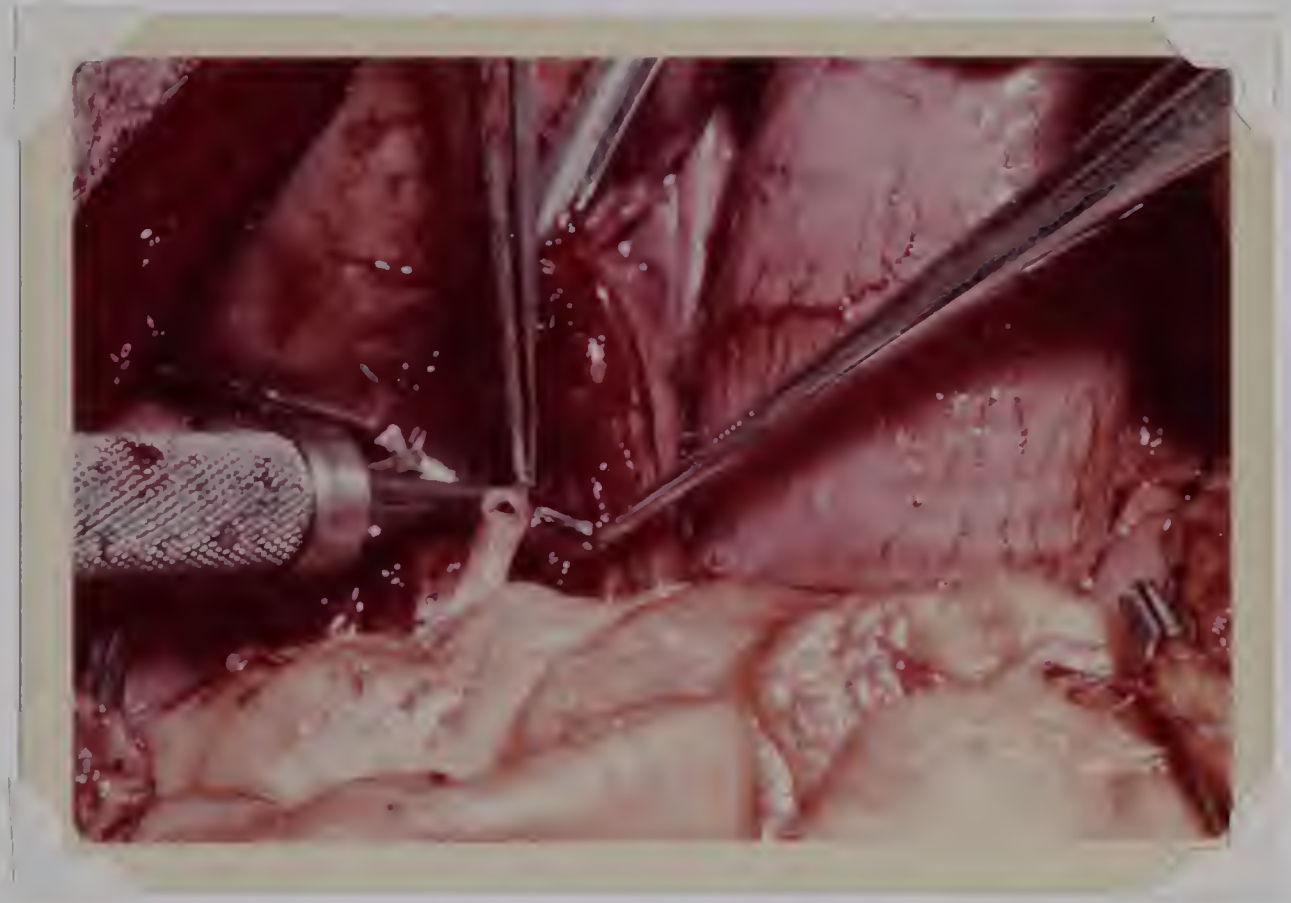


Fig. 10.-- Photograph showing arterial anastomosis using the pneumatic needle driver.

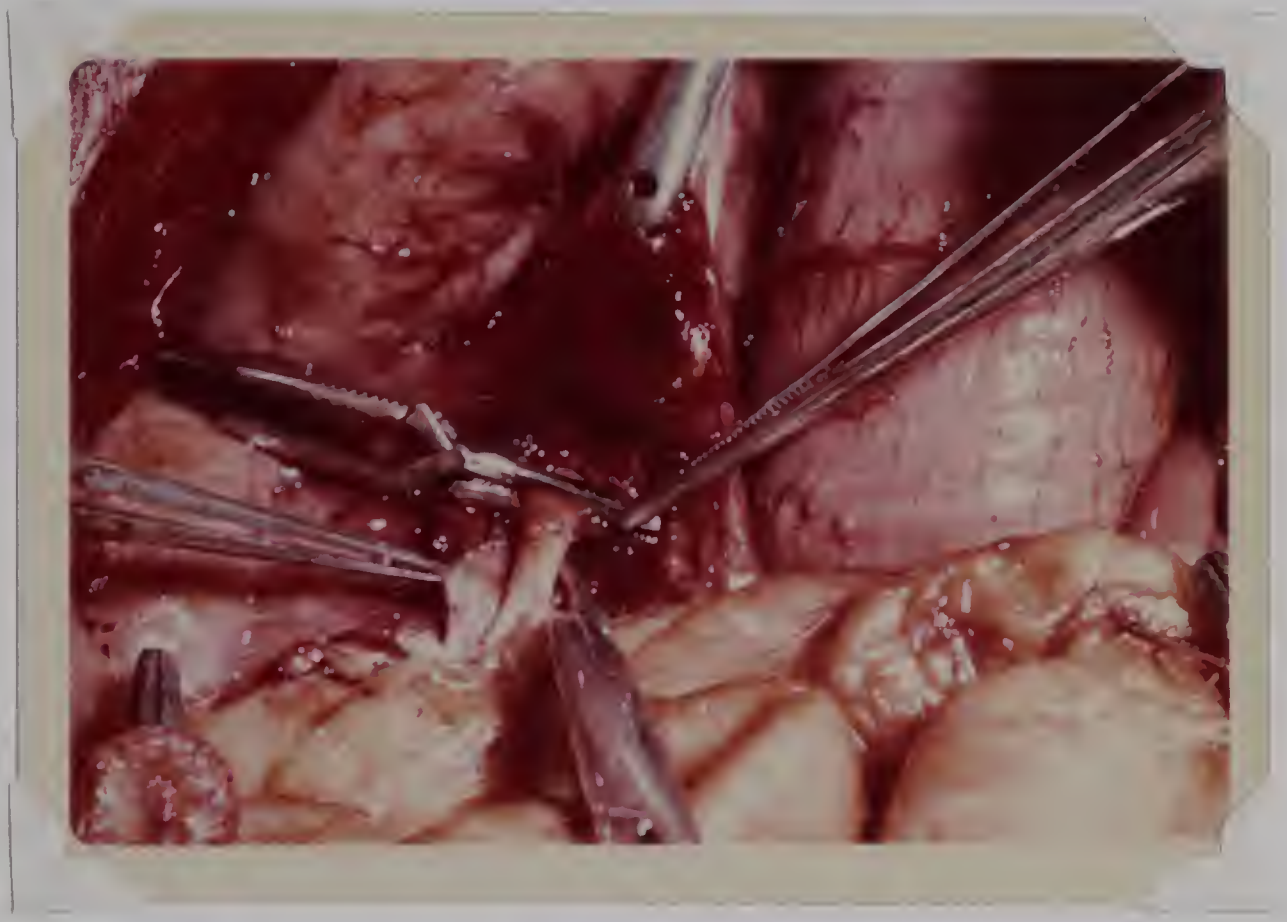


Fig. 11.-- Photograph showing completed arterial and venous anastomoses.

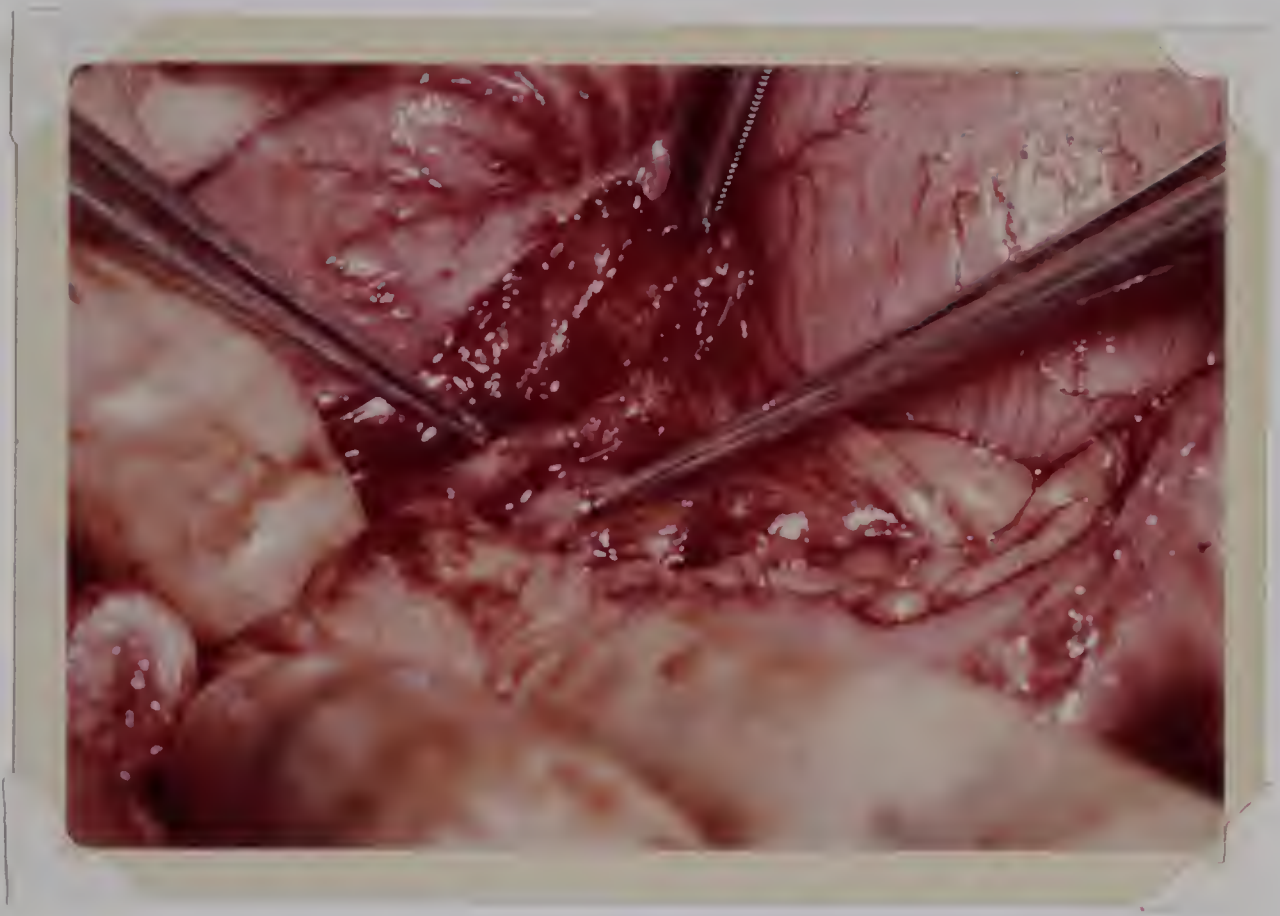


Fig. 12.-- Photograph showing completed arterial and venous anastomoses after release of the bulldog clamps.

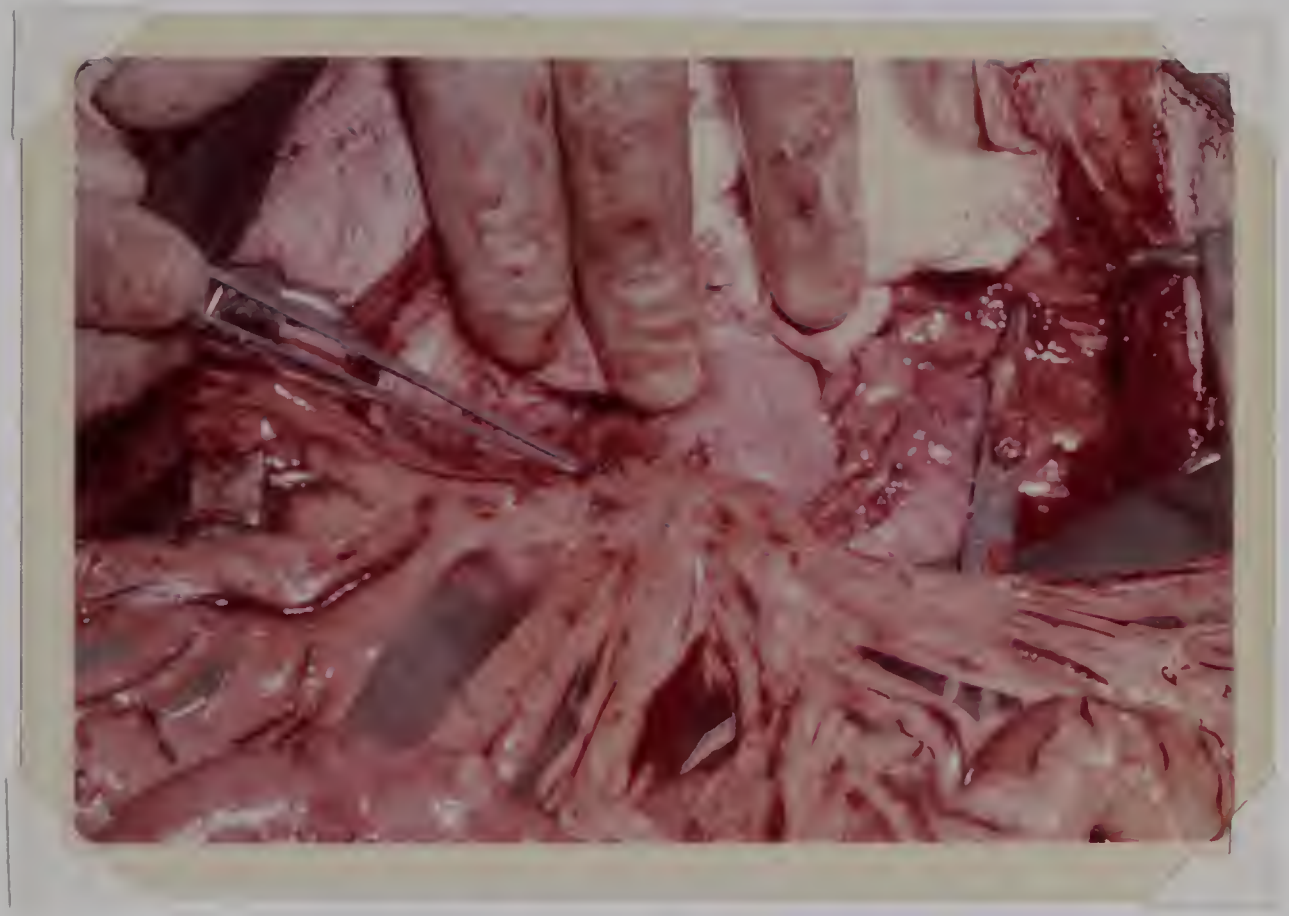


Fig. 13.-- Photograph showing completed lymph node anastomosis.

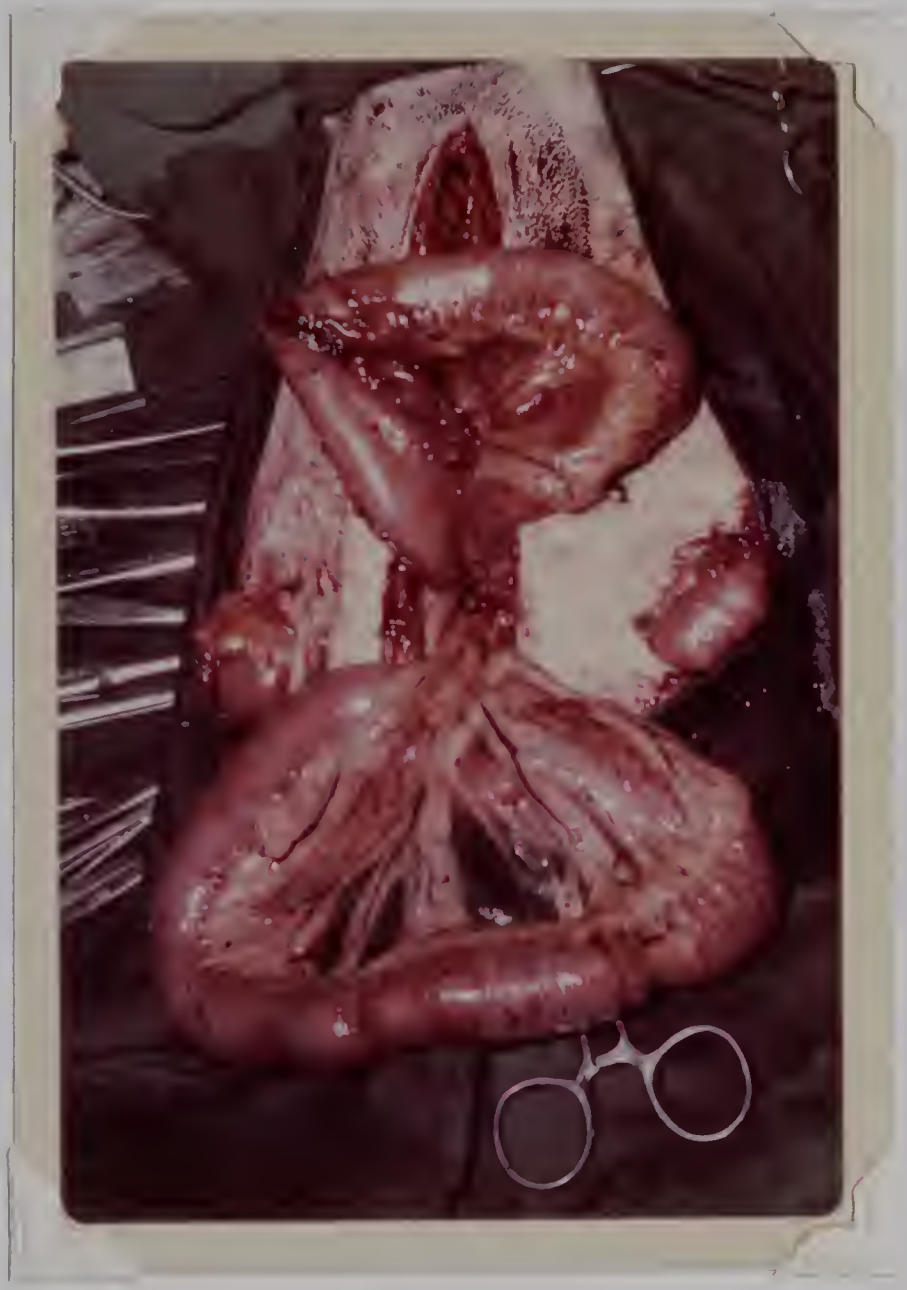


Fig. 14.-- Photograph showing anastomosis between the cut ends of dogs own bowel (above) and the transplanted loop of bowel with its ends exteriorised (below).

Fig. 15.-- Photograph showing appearance of stomas at the completion of the operation.

lactated Ringer's solution until all traces of blood were removed from the segment. The segment of bowel from the first dog was then transplanted into the second dog and vice versa. The mesenteric artery anastomosis was performed with a continuous 7-0 silk suture using the pneumatic needle driver.[3] A continuous 6-0 silk suture was used in the case of the mesenteric vein, and lymph nodes were anastomosed using interrupted 4-0 sutures.

The two ends of the isolated transplanted loop of intestine were exteriorised in the form of a Thiry-Vella loop fistula. The exteriorised bowel was sutured to the skin with 3-0 silk sutures.

The two free ends of the dogs own bowel (at the points of division mentioned previously) were then anastomosed using a single layer, inverting, closure of interrupted 4-0 silk sutures and the rent in the mesentery closed with interrupted 3-0 silk sutures.

The abdomen was then closed in layers. The peritoneum with a continuous 0 catgut suture, the anterior rectus sheath with interrupted 0 catgut sutures, and the skin with interrupted 0 silk sutures.

IX. Postoperative Management

After operation all dogs were kept in the operating room until they recovered from the anaesthetic and then returned to the animal quarters. They were given nothing by mouth for the first three days postoperation, except for dogs receiving azathioprine and prednisone, who were given these medications only, by mouth. During these three

initial days, dogs received daily intravenous administration of 1000 ml. of 5 percent dextrose-saline. They then received graduated feedings; liquid diet for two days, then special (semisolid) diet for two days before being returned to full diet. Stomas of the Thiry-Vella loop were carefully examined visually every day for signs of rejection.

Biopsies of Stomas

Biopsies of the exposed stomas were obtained at intervals and sent for histological study.

Autopsies

All dogs were subjected to postmortem examination. Sections were taken from the transplanted loop of bowel, the vascular anastomosis, and the mesenteric lymph node anastomosis, in addition to any other organ showing any pathologic change. These sections were studied histologically.

FOOTNOTES

[1]GESNER, B.M., and HOWARD, J.G. The isolation of lymphocytes and macrophages, Handbook of Experimental Immunology, ed. by D.M. Weir (Blackwell Scientific Publications, Oxford and Edinburgh, 1967), p. 1018-1022.

[2]GRAY, J.G., MONACO, A.P., WOOD, M.L., and RUSSELL, P.S. Studies on heterologous anti-lymphocytic serum in mice. I. In vitro and in vivo properties. J. Immunol., 96:217-228, 1966.

[3]SALMON, P.A. A new technique of microsuture for blood-vessel anastomosis. Brit. J. Surg., 55:58, 1968.

CHAPTER III

RESULTS AND CONCLUSIONS

I. In Vitro Tests

Antilymphocyte Serum

In the course of this series of experiments, eight batches of antilymphocyte sera were used. The in vitro characterisation indicated that in all cases dilutions of 1:256 produced agglutination of homologous lymphocytes.

Cytotoxic activity of the sera, as evidenced by Trypan blue staining, was positive.

'Pure' Antimacrophage Serum

Three batches of serum were used. Of these, the first two batches showed agglutination titres against peritoneal cells of 1:128. The final batch agglutinated peritoneal cells in dilutions of 1:256.

Pure antimacrophage serum showed only minimal cross reactivity with lymphocytes and dilutions of 1:1 and 1:2 produced only very weak agglutination with lymph node lymphocytes.

Cytotoxicity tests were positive in the case of peritoneal cells and negative when the purified antimacrophage serum was incubated with suspensions of lymph node lymphocytes.

'Impure' Antimacrophage Serum

The impure antimacrophage serum agglutinated peritoneal cells

in dilutions of 1:256 and lymph node lymphocytes in dilutions of 1:128. Cytotoxicity tests were positive for both peritoneal macrophages and lymph node lymphocytes.

II. Operation

The operation described gave very good immediate results. The implantation of the two grafts into their respective recipients were performed in sequence. The time taken to perform the blood vessel anastomoses was approximately 20 minutes for each animal. Thus the period of anoxia was 20 minutes for the first segment of intestine and 40 minutes for the second. The reestablishment of circulation was in all cases followed by immediate return of colour to the grafted segment and peristaltic action was resumed. We did not observe any complication which could be attributed to the period of anoxia.

During the first three postoperative days, our most troublesome complication was thrombosis, both arterial and venous (Figure 16). Approximately 40 percent of dogs receiving intestinal grafts either succumbed or were sacrificed because of this complication. These dogs were not included in our series. In an attempt to allay this complication, we heparinised two dogs after operation. Both dogs died from massive hemorrhage and, therefore this practice was discontinued.

III. Survival Studies

The dogs in the control group, who received no immunosuppressive therapy, survived for a mean of 7.4 days (Tables 1 and 6). These results

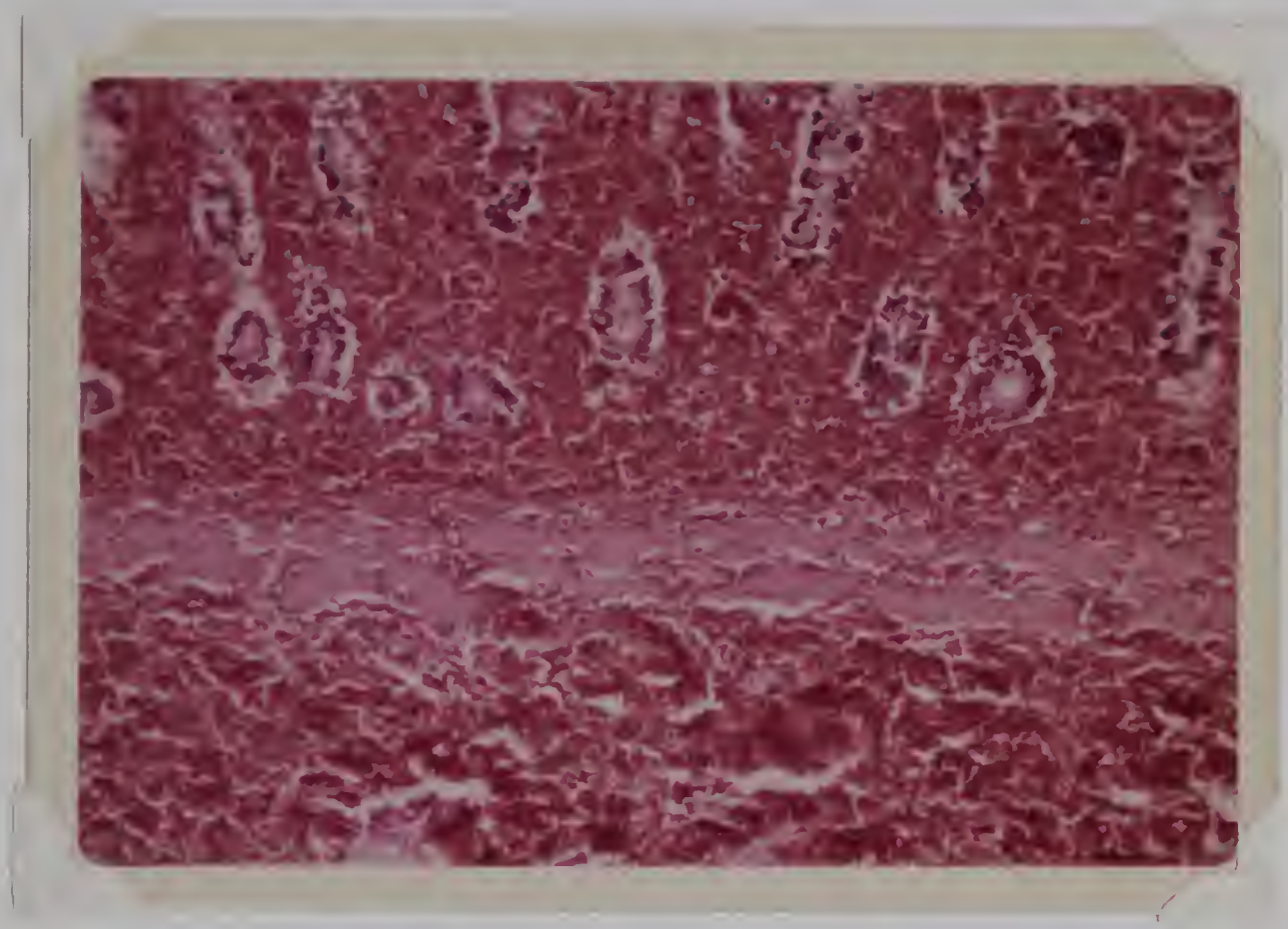


Fig. 16.-- Section of small intestine showing histologic appearance following venous thrombosis. Note intense congestion.

TABLE 1

CONTROL GROUP

Dog Number	Survival Time
F1713	6 days
G20	8 days
G52	7 days
G951	8 days
G819	8 days

Mean 7.40 days

±0.89

are in agreement with other published results.[1,2,3] Here there was healthy survival of grafts until the final day when there was a change in appearance of the stomas from a moist, glistening pink to a dry cyanotic (Figure 17) and finally a necrotic appearance. Necrosis was taken as the end point and dogs were sacrificed at this time. Microscopic study of grafts at this stage showed gross round cell infiltration with superimposed inflammatory and necrotic changes.

The second group of animals survived a mean of 13.4 days (Tables 2 and 6), demonstrating a significant prolongation of survival of grafts when antilymphocyte serum was the only immunosuppressive used. There have been no published results on the prolongation of survival of canine intestinal allografts with antilymphocyte serum. The grafts all looked healthy initially, but with the onset of rejection became necrotic and histologically showed round cell infiltration with dense inflammatory change and complete destruction of the mucosa (Figures 18 and 19).

The third group of animals received antimacrophage serum only. The mean survival was 6.83 days (Tables 3 and 6). Statistical comparison with the control group showed no significant difference. The administration of 'pure' antimacrophage serum produced no modification in the course of rejection of the grafts and the microscopic picture was typical of unmodified rejection.

Group IV animals received a combination of antilymphocyte serum and standard doses of azathioprine and prednisone. Dogs in this group showed healthy survival of grafts for a mean of 80 days (Tables 4 and 6).

Fig. 17.-- Photograph showing dry, cyanotic appearance of stoma of rejected transplanted loop of small intestine.

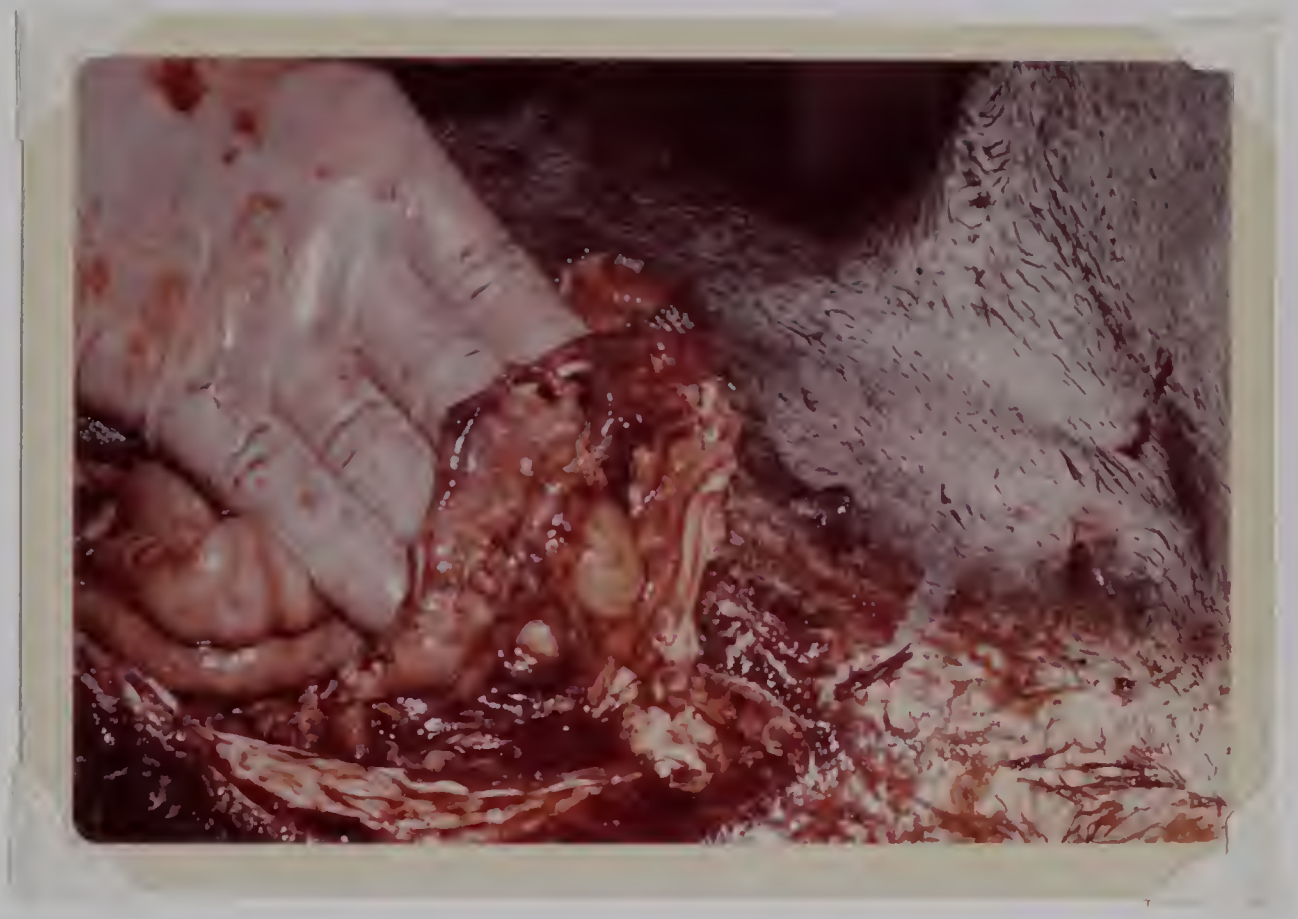


Fig. 18.-- Photograph showing necrotic appearance of a rejected small intestinal allograft.

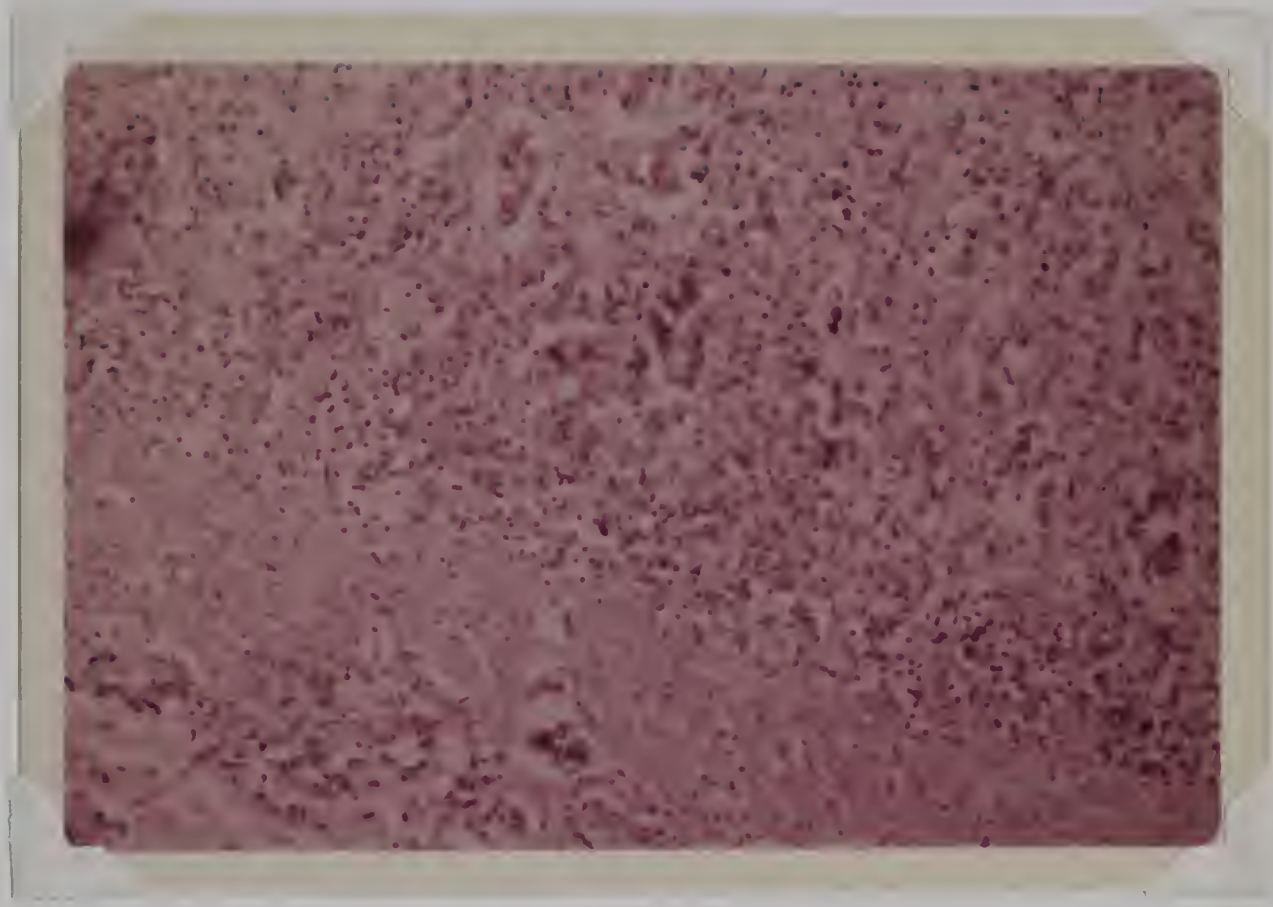


Fig. 19.-- Photograph showing histologic appearance of rejected small intestine. Note dense round cell infiltration and necrosis of mucosa.

TABLE 2

GROUP II - USING ALS ALONE AS IMMUNOSUPPRESSIVE

Dog Number	Survival Time
G1142	11 days
G1106	13 days
G1982	15 days
G571	14 days
E1025	14 days

Mean 13.4 days

± 1.52

ALS - Antilymphocyte serum

TABLE 3

GROUP III - USING 'PURE' AMS ONLY,
AS IMMUNOSUPPRESSIVE

Dog Number	Survival Time
G1936	8 days
G1928	7 days
G1903	5 days
G1922	7 days
H9	7 days
G1923	7 days

Mean 6.83 days

±0.98

AMS - Antimacrophage serum

TABLE 4

GROUP IV - USING ALS, AZATHIOPRINE AND PREDNISONE IN COMBINATION AS IMMUNOSUPPRESSIVES

Dog Number	Survival Time
G958	90 days
G1725	46 days
G1730	42 days
G1409	179 days*
G1661	52 days
G1697	10 days†

M = 80.0

SD = ±53.81

* - Still alive at the completion of the experiment

† - Died from an unrelated cause (refer to fig. 20). Not included in statistical analysis of results.

ALS - Antilymphocyte serum

One dog, which survived only 10 days, died following intussusception of the transplanted loop of bowel (Figure 20). This dog has not been included in the statistical evaluation of the results. In this group, the prolongation of survival is significant and one dog is still alive 179 days after receiving an intestinal allograft.

The most troublesome problem encountered in the dogs on prolonged courses of azathioprine, prednisone and antilymphocyte serum was the incidence of pulmonary infections which occurred in all animals in this series. These infections settled in the early stages with antibiotic therapy but confluent bronchopneumonia was eventually the cause of death in all of the animals in this series. At autopsy, transplanted loops of bowel in this group of dogs looked healthy, and histologically, the only abnormal finding was mild round cell infiltration (Figures 21 and 22).

The final group of dogs received an immunosuppressive regimen consisting of 5 ml. of antiserum, developed against crude peritoneal exudate, daily. These animals survived a mean of 29.33 days. In this group four animals survived 8 to 13 days and two survived 44 and 93 days respectively. It is difficult to explain the occurrence of this biomodal distribution of survival times. It may well represent a fortuitous tissue match. Further investigations will be necessary to determine this. As a result of the wide variation in survival times of the animals in this group, the results obtained are not statistically

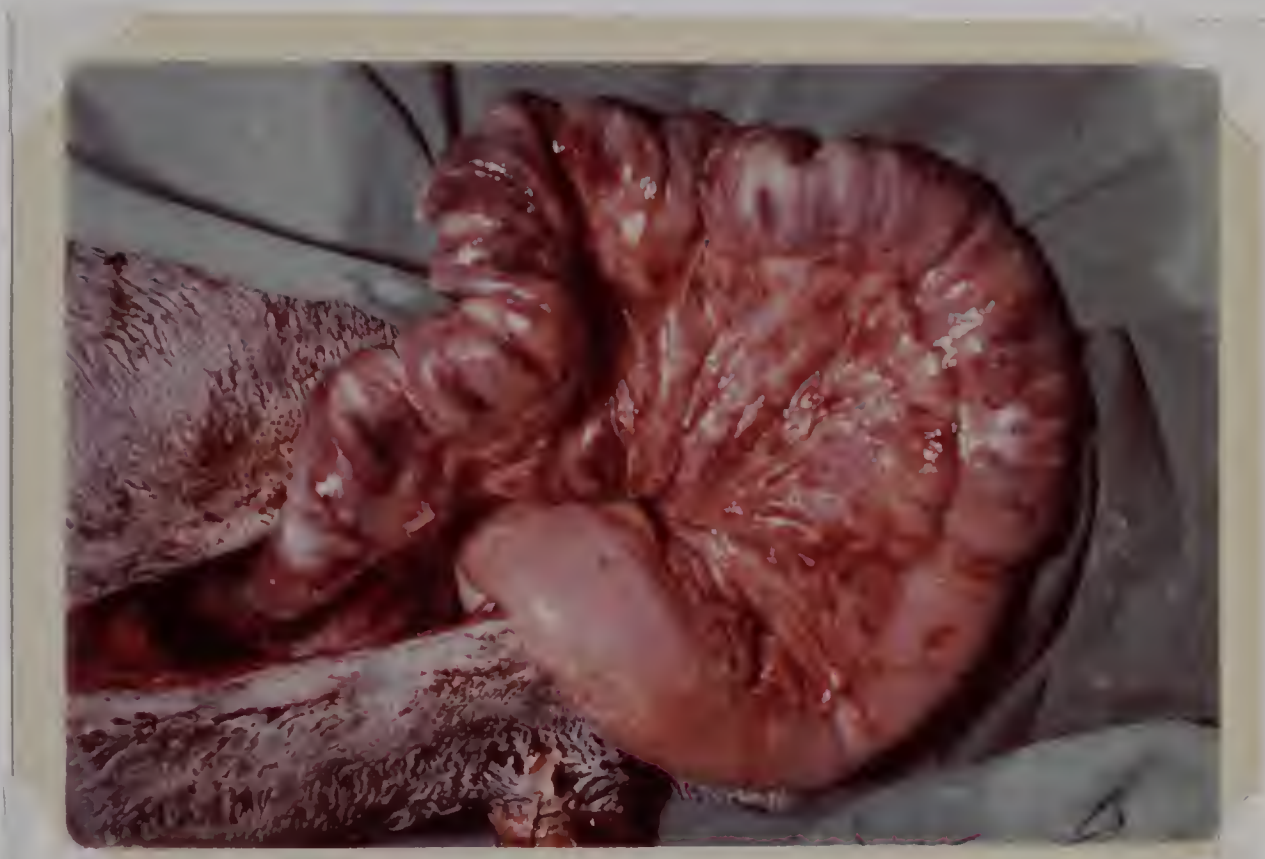


Fig. 20.-- Photograph showing intussusception in transplanted loop of small intestine.

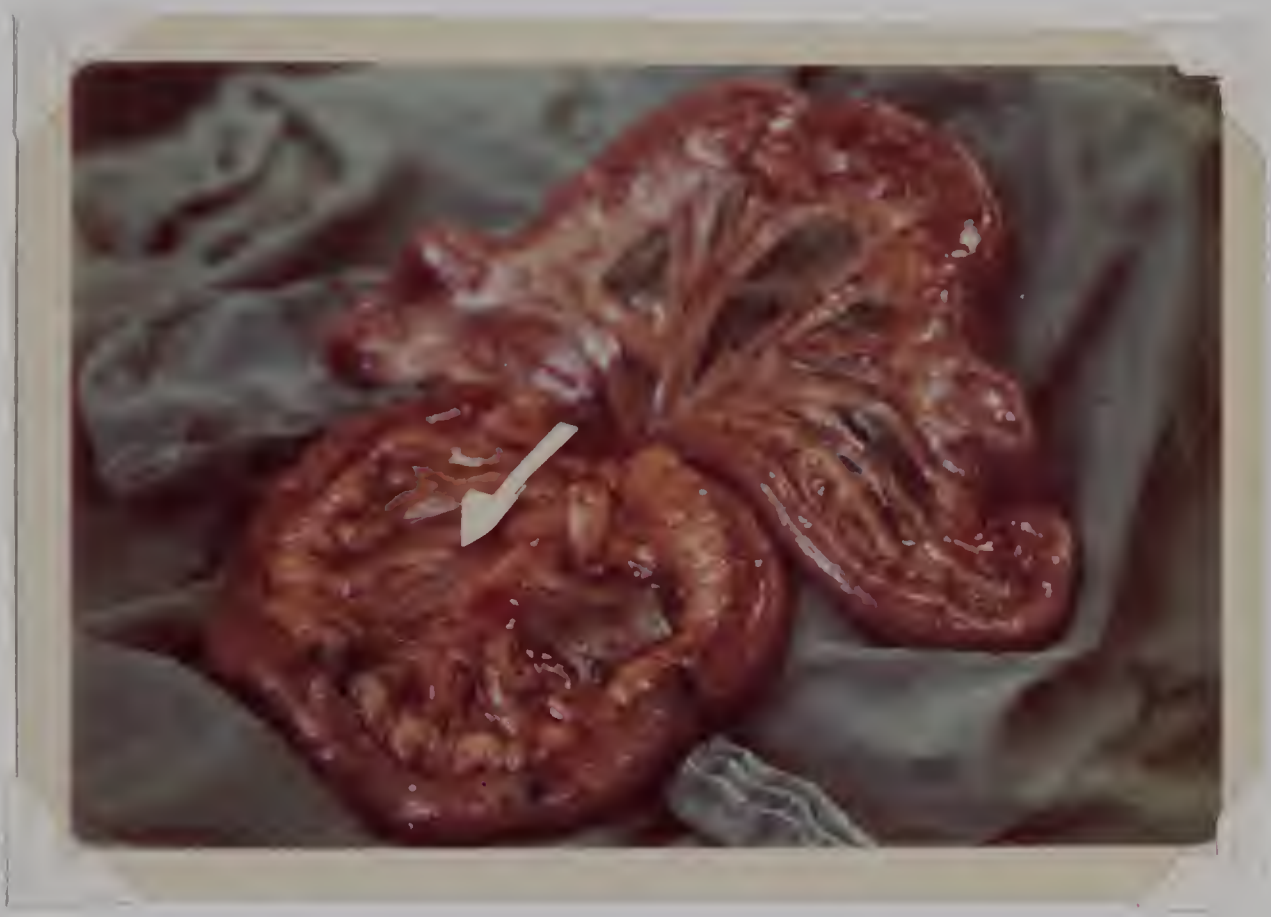


Fig. 21.-- Photograph showing normal appearance of the transplanted segment of small intestine (arrow) compared with a segment of dog's own small intestine (at top of photograph). This photograph was taken 93 days after the animal, G958, received an intestinal allograft. The immunosuppressive drugs used here were antilymphocyte serum, azathioprine and prednisone. The blue blotches on the transplanted segment represent sites of injection of methylene blue.

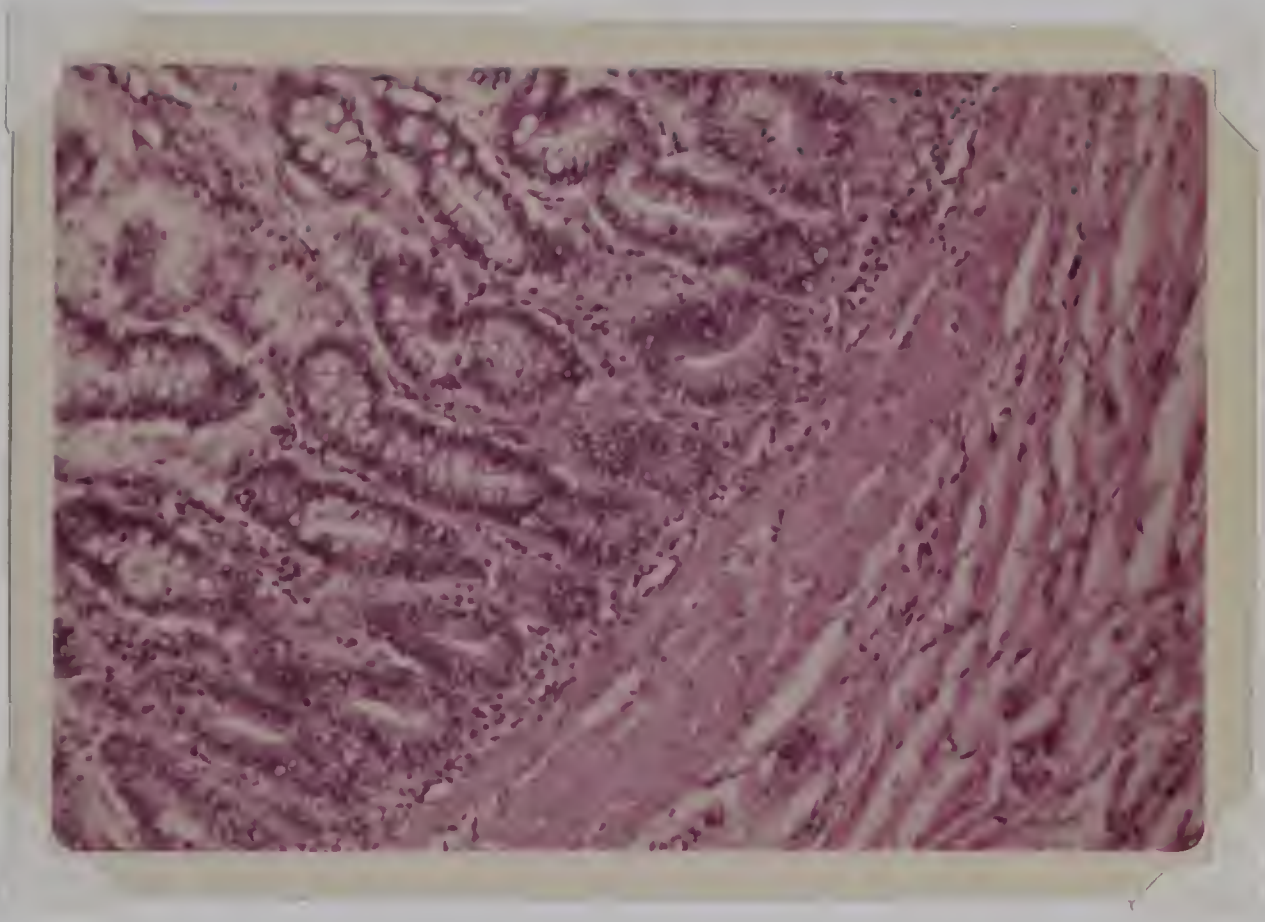


Fig. 22.-- Photograph of histologic section of allografted small intestine in dog G958, 93 days after transplantation. Note preservation of mucosa and only minimal round cell infiltration.

significant (Tables 5 and 6).

TABLE 5

GROUP V - USING 'IMPURE' AMS ONLY AS IMMUNOSUPPRESSIVE

Dog Number	Survival Time
H276	13 days
H379	44 days
H386	93 days
H377	9 days
H416	9 days
H474	8 days

M = 29.33

SD = 34.11

AMS - Antimacrophage serum

TABLE 6

STATISTICAL ANALYSIS OF RESULTS

Group	Number of Dogs	Mean Survival in Days	Standard Deviation	P Value*
I	5	7.4	0.89	
II	5	13.4	1.52	<0.001
III	6	6.83	0.98	>0.1
IV	6	80.0	53.81	<0.01
V	6	29.33	34.11	>0.05

*Statistical analysis of results was carried out using the Student's t test following

"Statistical Methods in Biology"
Norman T.S. Bailey
English Universities Press Ltd., London, 1959.

FOOTNOTES

[1]LILLEHEI, R.C., GOLDBERG, S., GOOT, B., and LONGERBEAN, J.K. Present status of intestinal transplantation. Ann. J. Surg., 105:58, 1963.

[2]PRESTON, F.W., MACALALAD, F., GRABER, R., JACKSON, E.J., and SPORN, J. Function and survival of jejunal homotransplants in dogs with and without immunosuppressive treatment. Transplantation, 3:224, 1965.

[3]STATE, D. Experimental studies in intestinal allografts. Surg. Clin. N. Amer., 49:547, 1969.

CHAPTER IV

SUMMARY AND DISCUSSION

In the series of experiments described, the following has been presented:

1) the technique used for obtaining a lymphocyte suspension and the preparation of an antiserum to this suspension,

2) the stimulation and collection of peritoneal cells and the purification of the crude macrophage suspension,

3) the preparation of antisera to:

   a) crude macrophage suspensions, and

   b) purified macrophage suspension,

4) the in vitro testing of the three types of antiserum used,

5) survival studies on dogs with intestinal allografts using three types of antiserum as immunosuppressives, alone, and in combination with standard immunosuppressive azathioprine and prednisone, and

6) the technique used in the preparation of canine intestinal allografts.

The method of preparation of lymphocyte suspensions involving manual homogenisation is crude and the cell counts obtained in these suspensions were lower than expected. More elaborate methods such as pressing lymph nodes through stainless steel mesh with pores of known diameter has resulted in higher cell counts.[1] The antilymphocyte serum obtained using our lymphocyte suspension has shown agglutination titres

of 1:256. Other workers[2] have obtained titres of up to 1:1024. These workers have developed their antisera in different animals. Rabbits and horses have been the most widely used species.[3] The goat has not gained much popularity as an experimental animal.

We made no attempt to sterilise our antisera owing to lack of facilities. However, apart from abscess formation at the site of injection in a few dogs, we encountered surprisingly few complications resulting from the use of unsterilised antisera.

The immunosuppressive potency of our antilymphocyte serum is demonstrated by the significant prolongation of survival of canine intestinal allografts obtained. There have been no published results on the effects of antilymphocyte serum on intestinal allografts but in the case of skin and other organ allografts, significant prolongation of survival has been obtained.[2,4,5,6,7]

The use of azathioprine and prednisone in the prolongation of survival of canine intestinal allografts has been reported by Preston et al[8] and by State.[9] These workers found that although survival was prolonged, the grafts were eventually transformed into fibrous tubes with loss of mucosa. Postmortem histological examination of grafts from the animals in our series has demonstrated good preservation of mucosa. Antilymphocyte serum thus seems to be a very useful addition to this regimen. We have conducted pilot studies on the function of these transplanted loops in dogs receiving combinations of antilymphocyte serum, azathioprine and prednisone and these so far have indicated

absorptive function no different from that of dogs with Thiry-Vella loops of their own bowel.

In the stimulation of peritoneal cells, intraperitoneal injection of olive oil was used. Other workers[10,11] have used glycogen and thioglycollate for this purpose. Here again, our cell counts were a little lower than those obtained using thioglycollate. However, olive oil was the most easily obtainable stimulate and it gave reasonable results. Culture of macrophages would have given a pure suspension.

The in vitro testing of our 'pure' antimacrophage serum showed agglutination of macrophage suspensions in dilutions of 1:128 - 1:256 with almost completely absent cross reaction with lymphocytes. Survival studies on 'pure' antimacrophage serum failed to demonstrate any immunosuppressive potency.

'Impure' antimacrophage serum, on the other hand, agglutinated both lymphocytes (dilutions of 1:256) and macrophages (1:128) demonstrating in vitro potency against both categories of cells. The results of our survival studies using this antiserum as immunosuppressive, are confusing. Four dogs survived 8 - 13 days, one dog survived 44 days and one dog is still alive at 93 days. When these results are all taken together, the result is not significant. However, in two dogs we have obtained survivals far greater than in any dogs treated with antilymphocyte serum. In these two dogs there has been good absorptive function of the transplanted loop. Further survival studies will be necessary to clarify this situation. Seeing that there was no prolongation of survival with 'pure' antimacrophage serum, one can postulate that any

immunosuppressive activity of the impure antimacrophage serum would be due to the antilymphocyte activity. A good method of ascertaining this would be to adsorb the 'impure' antimacrophage serum with a lymphocyte suspension and then to perform survival studies using the resulting antiserum.

The results of Dyminski and Argyris,[11] using an antiserum against peritoneal cells, suggest that antimacrophage serum has as good an immunosuppressive potency as antilymphocyte serum. It is difficult, however, to compare our results with theirs both because of the inconsistency of our own results and the difference in experimental model - they used skin allografts in mice.

Further investigation will be necessary to elucidate the rôle of the macrophage in rejection and the place of antimacrophage serum as an immunosuppressive.

FOOTNOTES

[1]GRAY, J.G., MONACO, A.P., WOOD, M.L., and RUSSELL, P.S. Studies on heterologous anti-lymphocytic serum in mice. I. In vitro and in vivo properties. J. Immunol., 96:217-228, 1966.

[2]ATAI, M., and KELLY, W.D. Prolongation of survival of canine kidney allografts with antilymphocyte serum. S. G. & O., 125:13, 1967.

[3]JAMES, K. The preparation and properties of antilymphocytic sera. Progr. Surg., 7:140-216, 1969.

[4]WOODRUFF, M.F.A., and ANDERSON, N.F. Effect of lymphocyte depletion by thoracic duct fistula and adminstration of antilymphocytic serum on the survival of skin homografts in rats. Nature (Lond.), 200:702, 1963.

[5]WOODRUFF, M.F.A., and ANDERSON, N.F. The effect of lymphocyte depletion by thoracic duct fistula and the administration of anti-lymphocytic serum on the survival of skin homografts in rats. Ann. N.Y. Acad. Sci., 120:119-128, 1964.

[6]STARZL, T.E., MARCHIORO, T.L., PORTER, K.A., IWASAKI, Y., and CERRILLI, C.J. The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal transplantation. Surg. Gynec. Obstet., 124:301-318, 1967.

[7]CLUNIE, G.J.A., NOLAN, B., JAMES, K., WATT, J.G., and WOODRUFF, M.F.A. Prolongation of canine renal allografts with anti-lymphocytic serum. Transplantation, 6:459-467, 1968.

[8]PRESTON, F.W., MACALALAD, F., GRABER, R., JACKSON, E.J., and SPORN, J. Function and survival of jejunal homotransplants in dogs with and without immunosuppressive treatment. Transplantation, 3:224, 1965.

[9]STATE, D. Experimental studies in intestinal allografts. Surg. Clin. N. Amer., 49:547, 1969.

[10]GESNER, B.M., and HOWARD, J.G. The isolation of lymphocytes and macrophages, Handbook of Experimental Immunology, ed. by D.M. Weir (Blackwell Scientific Publications, Oxford and Edinburg, 1967), p. 1018-1022.

[11]DYMINSKI, J.W., and ARGYRIS, B.F. Prolongation of allograft survival with antimacrophage serum. Transplantation, 8:595, 1969.

BIBLIOGRAPHY

[1]ARGYRIS, B.F. Role of macrophages in antibody production. Immune response to sheep red blood cells. J. Immunol., 99:774, 1967.

[2]ARGYRIS, B.F. Role of macrophages in immunological maturation. J. Exp. Med., 127:459, 1968.

[3]ARGYRIS, B.F., and ASKONAS, B. Mouse peritoneal cells: their ability to elicit or produce antibody after exposure to antigen. Immunology, 14:379, 1968.

[4]ARNASON, G.B., JANKOVIC, B.D., and WASKMAN, B.H. Role of the thymus in immune reactions in rats, Thymus in Immunobiology, ed. by R.A. Good and Ann Gabrielson (Hoeber [Harper Row], 1962), p. 492-400.

[5]ASKONAS, B.A., and RHODES, J.M. Immunogenicity of antigen-containing ribonucleic acid preparations from macrophages. Nature, 205: 470-474, 1965.

[6]ASPINALL, R., and MEYER, R. Effect of steroidal and surgical bursectomy and surgical thymectomy on the skin homograft reaction in chickens, Thymus in Immunobiology, ed. by R.A. Good and Ann Gabrielson (Hoeber [Harper Row], 1962), p. 376-394.

[7]ATAI, M., and KELLY, W.D. Prolongation of survival of canine kidney allografts with antilymphocyte serum. S. G. and O., 125:13, 1967.

[8]BILLINGHAM, R.E. Lymphocyte response to antigens. B. Efferent side of sensitisation arc: Evidence for cytopathic effects of lymphocytes. Transplantation, 5:976, 1967.

[9]BILLINGHAM, R.E., BRENT, L., MEDAWAR, P.B., and SPARROW, E.M. Quantitative studies on tissue transplantation immunity. 1. The survival times of skin homografts exchanged between members of different inbred strains of mice. Proc. Roy. Soc. Biol., 143:603, 1954.

[10]BLOOM, B.R., and BENNET, B. Migration inhibitory factor associated with delayed-type hypersensitivity. Fed. Proc., 27:13, 1968.

[11]BRENT, L., COURTENAY, T., and GOWLAND, G. Immunological reactivity of lymphoid cells after treatment with antilymphocytic serum. Nature (Lond.), 215:1461-1464, 1967.

[12]CLINE, M.J., and SWETT, V.C. The interaction of human monocytes and lymphocytes. J. Exp. Med., 128:1309, 1968.

[13]CLUNIE, G.J.A., NOLAN, B., JAMES, K., WATT, J.A., and WOODRUFF, M.F.A. Prolongation of canine renal allografts with antilymphocytic serum. Transplantation, 6:459-467, 1968.

[14]COOPER, M.D., PETERSON, R.D.A., SOUTH, M.A., and GOOD, R.A. Functions of the thymus system and bursa system in the chicken. J. Exp. Med., 123:75, 1966.

[15]DAVID, J.R. Macrophage migration. Fed. Proc., 27:6, 1968.

[16]DAVIES, A.J.S. Transplantation Rev., 1:43, 1969.

[17]DePETRIS, S., and KARLSBAND, G. Localization of antibodies by electron microscopy in developing antibody-producing cells. J. Cell Biol., 26:759, 1965.

[18]DRESSER, D.W. Effectiveness of lipid and lipidophilic substances as adjuvants. Nature, 191:1169, 1961.

[19]DRESSER, D.W. Adjuvanticity of vitamin A. Nature, 217:527, 1968.

[20]DYMINSKI, J.W., and ARGYRIS, B.F. Prolongation of allograft survival with antimacrophage serum. Transplantation, 8:595, 1969.

[21]EHRLICH, W.E. Lymphoid tissues: Their morphology and role in the immune response, Immunological Diseases, ed. by Samter, Little Brown Co., Boston, 1965, p. 251-277.

[22]FISHMAN, M. Antibody formation in vitro. J. Exp. Med., 114:837, 1967.

[23]FISHMAN, M., and ADLER, F.L. The role of macrophage RNA in the immune response. Cold Spring Harbor Symp. Quant. Biol., 32:343, 1968.

[24]FISHMAN, M., and ADLER, F.L. Antibody formation initiated in vitro. II. Antibody synthesis in X-irradiated recipients of diffusion chambers containing nucleic acid derived from macrophages incubated with antigen. J. Exp. Med., 117:595, 1963.

[25]FISHMAN, M., HAMMERSTROM, R.A., and BOND, V.P. In vitro transfer of macrophages RNA to lymph node cells. Nature (Lond.), 198:549, 1963.

[26]FITCH, F.W., and WISSLER, R.W. The histology of antibody production, Immunological Diseases, ed. by Samter, Little Brown Co., Boston, 1965, p. 65-86.

[27]FRIEDMAN, A.P., STRAVISKI, A.B., and SOLOMON, J.M. Induction in vitro of antibodies to phage P-2: antigens in RNA extract employed. Science, 149:1106, 1965.

[28]FREUND, J. Advances Tuberc. Res., 7:130, 1956.

[29]GALL, D. The adjuvant activity of aliphatic nitrogenous bases. Immunology, 11:369, 1966.

[30]GALLILY, R., and FELDMAN, M. The role of macrophages in the induction of antibody in X-irradiated animals. Immunology, 12:197, 1967.

[31]GESNER, B.M., and HOWARD, J.G. The isolation of lymphocytes and macrophages, Handbook of Experimental Immunology, ed. by D.M. Weir (Blackwell Scientific Publications, Oxford and Edinburg, 1967), p. 1018-1022.

[32]GINSBERG, H. Graft versus host reaction in tissue culture. 1. Lysis of monolayers of embryo mouse cells from strains differing in the H-2 histocompatibility locus by rat lymphocytes sensitised in vitro. Immunology, 14:621, 1968.

[33]GOLUB, E.S., and WEIGLE, W.O. Studies on the induction of immunologic unresponsiveness. 1. Effects of endotoxin and phytohemagglutinin. J. Immunol., 98:1241, 1967.

[34]GOOT, B., LILLEHEI, R.C., and MILLER, F.A. Homografts of small bowel. Surg. Forum, 10:193, 1959.

[35]GOTTLIEB, A.A. Antigens, RNAs, and macrophages. J. Reticuloendothelial Soc., 5:270, 1968.

[36]GOWANS, J., and McGREGGOR, D. The immunological activities of lymphocytes. Prog. Allergy, 9:1, 1965.

[37]GOWANS, J.L., McGREGGOR, D.J., and COWEN, D.M. The role of small lymphocytes in the rejection of homografts of skin: The Immunologically Competent Cell: Its Nature and Origin, (Ciba Foundation Study Group No. 16), ed. by G.E.W. Wolstenholme and J. Knight (Little, Brown and Company, Boston, 1963), p. 20-37.

[38]GRANGER, G.A., and WEISER, R.S. Homograft target cells: Specific destruction in vitro by contact interaction with immune macrophages. Science, 145:1427, 1964.

[39]GRANGER, G.A., and WEISER, R.S. Homograft target cells: Contact destruction in vitro by immune macrophages. Science, 151:97, 1966.

[40]GRAY, J.G., MONACO, A.P., WOOD, M.L., and RUSSELL, P.S. Studies on heterologous anti-lymphocytic serum in mice. I. In vitro and in vivo properties. J. Immunol., 96:217-228, 1966.

[41]GREAVES, M.F., TORRIGIANI, G., and ROITT, I.M. Blocking of the lymphocyte receptor site for cell mediated hypersensitivity and transplantation reactions by anti-light chain sera. Nature (Lond.), 222:885, 1969.

[42]GUTTMAN, R.D., CARPENTER, C.B., LINDQUIST, R.R., and MERRILL, J.P. Renal transplantation in the inbred rat. III. A study of heterologous and antithymocyte sera. J. Exp. Med., 126:1099-1127, 1967.

[43]HARRIS, T.N., HUMMELER, K., and HARRIS, S. Electron microscopic observations on antibody-producing lymph node cells. J. Exp. Med., 123:161-172, 1966.

[44]HERSH, E.M., and HARRIS, J.E. Macrophage-lymphocyte interaction in the antigen-induced blastogenic response of human peripheral blood leukocytes. J. Immunol., 100:1184, 1968.

[45]HOLM, G., PERLMANN, P., and WERNER, B. Phytoaemeagglutinin-induced cytotoxic action of normal lymphoid cells on cells in tissue culture. Nature (Lond.), 203:841, 1964.

[46]INDERBITZIN, T. The relationship of lymphocytes, delayed cutaneous allergic reactions and histamine. Int. Arch. Allergy, 8:150-159, 1956.

[47]IWASAKI, Y., PORTER, K.A., AMEND, J.R., MARCHIORD, T.L., ZUHLKE, V., and STARZL, T.E. The preparation and testing of horse anti-dog and anti-human anti-lymphoid plasma or serum and its protein fractions. Surg. Gynec. Obstet., 124:1-24, 1967.

[48]JAMES, K. Anti-lymphocytic antibody-A review. Clin. Exp. Immunol., 2:615-631, 1967.

[49]JAMES, K. The preparation and properties of antilymphocytic sera. Progr. Surg., 7:140-216, 1969.

[50]JAMES, K., and MEDAWAR, P.B. Characterisation of antilymphocyte antibody. Nature (Lond.), 214:1052-1053, 1967.

[51]JEHN, U.W., MUSHER, D.M., and WEINSTEIN, L. The effect of anti-macrophage serum on macrophage-lymphocyte interaction in vitro. Proc. Soc. Exp. Biol. Med., 134:241, 1970.

[52]JEEJEEBHOY, H.F. Studies on the mode of action of heterologous antilymphocyte plasma. I. A comparison of the immunosuppressive properties of dog and rabbit anti-rat lymphocyte plasma. Transplantation, 5:273, 1967.

[53]KOLSCH, E., and MITCHISON, N.A. The subcellular distribution of antigen in macrophages. J. Exp. Med., 128:1059, 1968.

[54]LEVEY, R.H., and MEDAWAR, P.B. Some experiments on the actions of antilymphoid antisera. Ann. N.Y. Acad. Sci., 129:164-117, 1966.

[55]LEVEY, R.H., and MEDAWAR, P.B. Nature and mode of antilymphocytic serum. Proc. Nat. Acad. Sci. (USA), 56:1130-1137, 1966.

[56]LILLEHEI, R.C., GOLDBERG, S., GOOT, B., and LONGERBEAN, J.K. Present status of intestinal transplantation. Ann. J. Surg., 105:58, 1963.

[57]LILLEHEI, R.C., GOOT, B., and MILLER, F.A. Physiological response of small bowel of the dog to ischemia including prolonged in vitro preservation of the bowel with successful replacement and survival. Ann. Surg., 105:543, 1959.

[58]LILLEHEI, R.C., IDEZUKI, Y., FEEMSTER, J.A., DIETZMAN, R.H., KELLY, W.D., MERKEL, F.K., GOETZ, F.C., LYONS, A.W., and MANAX, W.G. Transplantation of stomach intestine and pancreas - experimental and clinical observations. Surgery, 62:721, 1967.

[59]LOEB, L. The biological basis of individuality. Springfield Thomas, 1945.

[60]MARCHESI, V.T., and GOWANS, J.L. The migration of lymphocytes through the endothelium of venules in lymph nodes: An electron microscopic study. Proc. Roy. Soc. (Biol.), 159:283, 1964.

[61]MARTINEZ, C., DALMASSO, A.P., and GOOD, R.A. Homotransplantation of normal and neoplastic tissue in thymectomised mice, Thymus in Immunobiology, ed. by R.A. Good and Ann Gabrielson (Hoeber [Harper Row], 1962), p. 465-477.

[62]MEDAWAR, P.B. The behavior and fate of skin autografts and skin homografts in rabbits. J. Anat., 78:176, 1944.

[63]MERRITT, K., and JOHNSON, A.G. Studies on the adjuvant action of bacterial endotoxins on antibody formation. V. The influence of endotoxin and 5-Fluoro-2 Deoxyuridine on the primary antibody response of the BALB mouse to a purified protein antigen. J. Immunol., 91:266, 1963.

[64]METCHINKOFF, E. Ann. Inst. Past., 13:737, 1899.

[65]MILLER, J.F.A.P. Immunological significance of the thymus, Advances In Immunology, vol. 2, ed. by W. Taliaferro and J.H. Humphrey (Academic Press, New York and London, 1962).

[66]MILLER, J.F.A.P. The thymus and transplantation immunity. Brit. Med. Bull., 21:111, 1965.

[67]MILLER, J.F., and MITCHELL, G.F. Transplantation Rev., 1:1, 1969.

[68]MITCHISON, N.A. Passive transfer of transplantation immunity. Nature (Lond.), 171:267, 1953.

[69]MITCHISON, N.A. Induction of immunological paralysis in two zones fo dosage. Proc. Roy. Soc. Lond. B, 161:275, 1965.

[70]MOISER, D.E. Cell interactions in the primary response in vitro: a requirement for specific cell clusters. J. Exp. Med., 129:351, 1969.

[71]MOISER, D.E. A requirement for two cell types for antibody formation in vitro. Science (Washington), 158:1575, 1967.

[72]MOISER, D.E., and COPPLESON, L.W. A three cell interaction required for the induction of a primary immune response in vitro. Proc. Nat. Acad. Sci. (USA), 61:542, 1968.

[73]MÖLLER, E. Contact-induced cytotoxicity by lymphoid cells containing foreign isoantigens. Science, 147:873, 1965.

[74]MONACO, A.P., ABBOTT, W.N., OTHERSON, H.B., SIMMONS, R.L., WOOD, M.L., FLAX, M.H., and RUSSELL, P.S. Antiserum to lymphocytes: Prolonged survival of canine renal allografts. Science, 153: 1264-1267, 1966.

[75]MONACO, A.P., WOOD, M.L., and RUSSELL, P.S. Studies on heterologous antilymphocyte serum in mice. III. Immunologic tolerance and chimerism produced across the H-2 locus with adult thymectomy and antilymphocyte serum. Ann. N.Y. Acad. Sci. Wash., 129:190-209, 1966.

[76]MONACO, A.P., WOOD, M.L., and RUSSELL, P.S. Adult thymectomy: effect on recovery from immunologic depression in mice. Science, 149:432-435, 1965.

[77]McGREGGOR, D.J. Bone marrow origin of immunologically competent lymphocytes in the rat. J. Exp. Med., 125:953, 1968.

[78]NAGAYA, H., and SIEKER, H.O. Allograft survival: Effect of antiserums to thymus glands and lymphocytes. Science, 150:1181-1182, 1965.

[79]NELSON, D.S. Macrophages in immunity, Frontiers in Biology Series, ed. by A. Neuberger and E.L. Tatum (North Holland Publishing Company, Amsterdam, London, 1969), p. 41.

[80]PERSALL, N.N., and WEISER, R.S. The macrophage in allograft immunity. II. Passive transfer with immune macrophages. J. Reticuloendothelial Soc., 5:121, 1968.

[81]PERSALL, N., and WEISER, R. The macrophage in allograft immunity. 1. Effects of silica as a specific macrophage toxin. J. Reticuloendothelial Soc., 5:107-120, 1968.

[82]PICHLMAYR, R., BRENDEL, W., and ZENKER, R. Production and effect of heterologous anti-canine lymphocyte serum. Surgery, 61:774, 1967.

[83]PIERCE, C.W. Immune responses in vitro. I. Cellular requirements for the immune response by nonprimed and primed spleen cells in vitro. J. Exp. Med., 130:345-363, 1969.

[84]PRESTON, F.W., MACALALAD, F., GRABER, R., JACKSON, E.J., and SPORN, J. Function and survival of jejunal homotransplants in dogs with and without immunosuppressive treatment. Transplantation, 3: 224, 1965.

[85]RUSSELL, P.S., and MONACO, A.P. Heterologous anti-lymphocyte sera and some of their effects. Transplantation, 5:1086-1099, 1967.

[86]RUSSEL, P.J., and WINN, H.J. Transplantation. New Eng. J. Med., 282:848, 1970.

[87]SALMON, P.A. A new technique of microsuture for blood vessel anastomosis. Brit. J. Surg., 55:58, 1968.

[88]SCHONENBERG, M.D., MUMOW, V.R., MOORE, R.D., and WEISBERGER, A.S. Cytoplasmic interaction between macrophage and lymphocytic cells in antibody synthesis. Science (Washington), 143:964, 1967.

[89]SELL, S. Studies on rabbit lymphocytes in vitro v. the induction of blast transformation with sheep antisera to rabbit IgG subunits. J. Exp. Med., 125:289, 1967.

[90]SELL, S., and GELL, P.G.H. Studies on rabbit lymphocytes in vitro. I. Stimulation of blast transformation with anti-allotype serum. J. Exp. Med., 122:423, 1965.

[91]SPITZNAGEL, J.K., and ALLISON, A.C. Mode of action of adjuvants. Retinol and other lysosome labilising agents as adjuvants. J. Immunol., 104:119, 1970.

[92]STARZL, T.E., MARCHIORO, T.L., PORTER, K.A., IWASAKI, Y., and CERRILLI, C.J. The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal transplantation. Surg. Gynec. Obstet., 124:301-318, 1967.

[93]STARZL, T.E., PORTER, K.A., IWASAKI, Y., MARCHIORO, T.L., and KASHIWAGI, N. The use of heterologous antilymphocyte globulin in human renal homotransplantation, Antilymphocytic Serum, (Ciba Foundation Study Group No. 29), ed. by G.E.W. Wolstenholme and M. O'Connor (Little, Brown and Company, Boston, 1967), p. 4-40.

[94]STATE, D. Experimental studies in intestinal allografts. Surg. Clin. N. Amer., 49:547, 1969.

[95]THORABECKE, G.J., and BENACERRAF, B. The reticulo-endothelial system and immunological phenomena. Prog. Allergy, 6:559, 1962.

[96]UNANUE, E.R. Properties and some uses of antimacrophage antibodies. Nature, 218:36, 1968.

[97]UNANUE, E.R., and ASKONAS, B.A. Persistence of immunogenicity of antigen after uptake by macrophages. J. Exp. Med., 127:915, 1968.

[98]UNANUE, E.R., and ASKONAS, B.A. Two functions of macrophages and their role in immune responses. J. Reticulo-endothelial Soc., 4:440, 1967.

[99]WAKSMAN, B.H. The pattern of rejection in rat skin homografts and its relation to the vascular network. Lab. Invest., 12:46, 1963.

[100]WAKSMAN, H.B., ARBUOYS, S., and ARNASON, B.C. The use of specific lymphocyte anti-sera to inhibit hypersensitive reactions of the delayed type. J. Exp. Med., 114:997-1021, 1961.

[101]WAKSMAN, B.H., ARNASON, B.G., and JANKOVIC, B.D. Changes in the lymphoid organs of rats thymectomised at birth. Federation Proc., 21(Part 2):274, 1962.

[102]WEIGLE, W., DIXON, F.J., DEICHMULLER, M.D. Effect of mycobacteria on antibody production induced by water in oil emulsions of soluble protein antigens. Proc. Soc. Exp. Biol. Med., 105:535, 1960.

[103]WEINER, J., SPIRO, D., and RUSSELL, P. An electron microscopic study of the homograft reaction. Am. J. Pathol., 44:319, 1964.

[104]WISSLER, R., FITCH, F., and LAVIN, M. The relations of the R.E.S. to infection and immune reactions. The R.E.S. in antibody formation. Ann. N. Y. Acad. Sci., 88:134, 1960.

[105]WOODRUFF, M.F.A. The transplantation of tissues and organs, (Thomas Springfield, Illinois, 1966).

[106]WOODRUFF, M.F.A., and ANDERSON, N.F. Effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocytic serum on the survival of skin homografts in rats. Nature (Lond.), 200:702, 1963.

[107]WOODRUFF, M.F.A., and ANDERSON, N.F. The effect of lymphocyte depletion by thoracic duct fistula and the administration of anti-lymphocytic serum on the survival of skin homografts in rats. Ann. N. Y. Acad. Sci., 120:119-128, 1964.

[108]WOODRUFF, M.F.A., ANDERSON, N.F., and ABAZA, H.M. Experiments with antilymphocytic serum, Bristol Symposium on the Lymphocyte in Immunology and Haemopoisesis, ed. by Edward Arnold (London, 1966), p. 286.

[109]WOODRUFF, M.F.A., JAMES, K., ANDERSON, N.F., and REID, B.L. Comparison of in vivo and in vitro properties of ALS; in Ciba Study Group on Antilymphocyte Serum, p. 57-58, (Churchill, London, 1967a).

APPENDIX I

In the data presented, two dogs remained alive at the completion of the experiment. The animal, G1409, has been on an immunosuppressive regimen of antilymphocyte serum, azathioprine, and prednisone while the animal, H386, has received 'impure' antimacrophage serum only. These dogs are still alive and well - G1409 having now survived 325 days and H386, 239 days. Since the completion of the experiment, the transplanted loop of bowel has been placed in continuity with the dog's own bowel in both cases. Both dogs have gained weight and appear completely healthy.

APPENDIX II

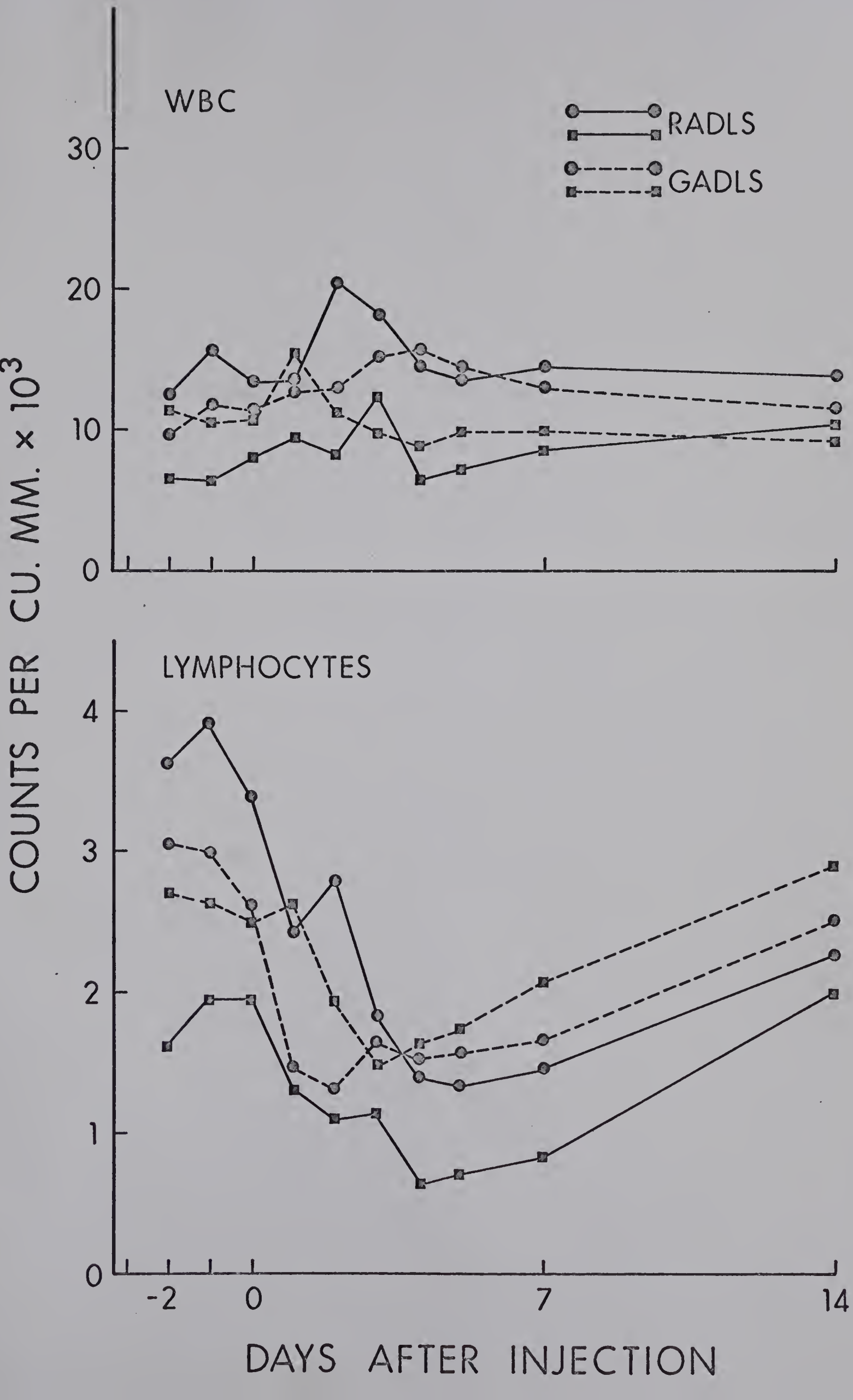
Control studies were conducted in four small healthy dogs to determine the effect of antilymphocyte serum on absolute lymphocyte counts.[1] Moderate lymphopenia was noted, which lasted ten days. Lymphocyte counts tended to return to normal after this time. However, the total leukocyte count seemed unaffected. These results are demonstrated in the accompanying figure (23) which represents the effects of antilymphocyte serum on circulating lymphocyte and absolute leukocyte levels in healthy dogs.

R.A.D.L.S. represents rabbit-antidog lymphocyte serum.

G.A.D.L.S. represents goat-antidog lymphocyte serum.

---

[1]SALMON, P.A., LEE, K.K., and BOISVERT, D.P.J. Prolongation of canine intestinal allograft survival with and without antilymphocyte serum, azathioprine and prednisone. Unpublished data, 1970.


WBC
RADLS
GADLS
30
20
10
0
CU. MM. x 10^3
LYMPHOCYTES
4
3
2
1
0
COUNTS PER
-2
0
7
14
DAYS AFTER INJECTION